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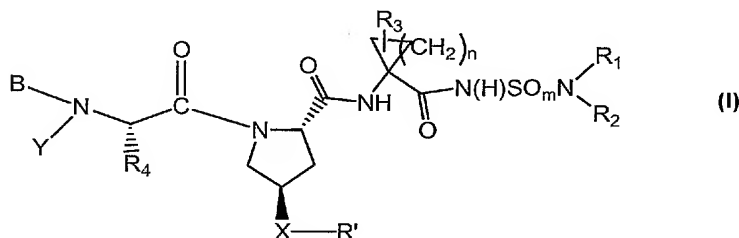
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(54) Title: HEPATITIS C VIRUS INHIBITORS



(57) Abstract: Compounds are disclosed having general formula (I), wherein R₁, R₂, R₃, R₄, R', B, Y and X are described in the description. Compositions comprising the compounds and methods for using the compounds to inhibit HCV are also disclosed.



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HEPATITIS C VIRUS INHIBITORS

FIELD OF THE INVENTION

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The present invention is generally directed to antiviral compounds, and more specifically directed to compounds which inhibit the functioning of the NS3 protease (also referred to herein as "serine protease") encoded by Hepatitis C virus (HCV), compositions comprising such compounds and methods for inhibiting the functioning of the NS3 protease.

10

BACKGROUND OF THE INVENTION

HCV is a major human pathogen, infecting an estimated 170 million persons worldwide - roughly five times the number infected by human immunodeficiency virus type 1. A substantial fraction of these HCV infected individuals develop serious progressive liver disease, including cirrhosis and hepatocellular carcinoma. (Lauer, G. M.; Walker, B. D. *N. Engl. J. Med.* (2001), 345, 41-52).

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Presently, the most effective HCV therapy employs a combination of alpha-interferon and ribavirin, leading to sustained efficacy in 40% of patients. (Poynard, T. et al. *Lancet* (1998), 352, 1426-1432). Recent clinical results demonstrate that pegylated alpha-interferon is superior to unmodified alpha-interferon as monotherapy (Zeuzem, S. et al. *N. Engl. J. Med.* (2000), 343, 1666-1672). However, even with experimental therapeutic regimens involving combinations of pegylated alpha-interferon and ribavirin, a substantial fraction of patients do not have a sustained reduction in viral load. Thus, there is a clear and long-felt need to develop effective therapeutics for treatment of HCV infection.

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HCV is a positive-stranded RNA virus. Based on a comparison of the deduced amino acid sequence and the extensive similarity in the 5' untranslated region, HCV has been classified as a separate genus in the Flaviviridae family. All members of the Flaviviridae family have enveloped virions that contain a positive

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stranded RNA genome encoding all known virus-specific proteins via translation of a single, uninterrupted, open reading frame.

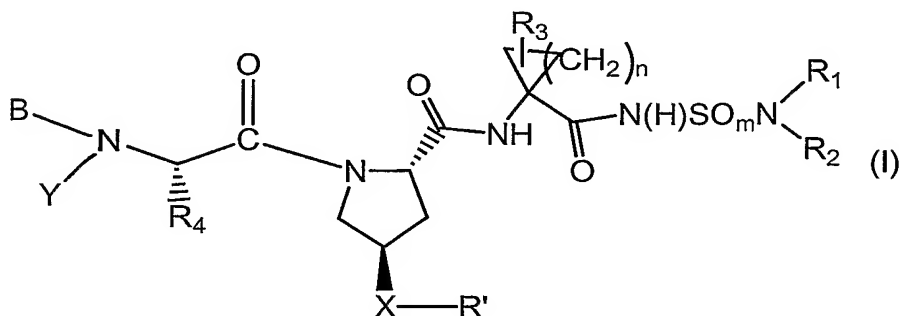
Considerable heterogeneity is found within the nucleotide and encoded amino acid sequence throughout the HCV genome. At least six major genotypes have been characterized, and more than 50 subtypes have been described. The major genotypes of HCV differ in their distribution worldwide, and the clinical significance of the genetic heterogeneity of HCV remains elusive despite numerous studies of the possible effect of genotypes on pathogenesis and therapy.

The single strand HCV RNA genome is approximately 9500 nucleotides in length and has a single open reading frame (ORF) encoding a single large polyprotein of about 3000 amino acids. In infected cells, this polyprotein is cleaved at multiple sites by cellular and viral proteases to produce the structural and non- structural (NS) proteins. In the case of HCV, the generation of mature non-structural proteins (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) is effected by two viral proteases. The first one is believed to cleave at the NS2-NS3 junction; the second one is a serine protease contained within the N-terminal region of NS3 and mediates all the subsequent cleavages downstream of NS3, both in cis, at the NS3-NS4A cleavage site, and in trans, for the remaining NS4A- NS4B, NS4B-NS5A, NS5A-NS5B sites. The NS4A protein appears to serve multiple functions, acting as a cofactor for the NS3 protease and possibly assisting in the membrane localization of NS3 and other viral replicase components. The complex formation of the NS3 protein with NS4A seems necessary to the processing events, enhancing the proteolytic efficiency at all of the sites. The NS3 protein also exhibits nucleoside triphosphatase and RNA helicase activities. NS5B is a RNA-dependent RNA polymerase that is involved in the replication of HCV.

Among the compounds that have demonstrated efficacy in inhibiting HCV replication, as selective HCV serine protease inhibitors, are the peptide compounds disclosed in U.S. Patent No. 6,323,180.

SUMMARY OF THE INVENTION

The present invention relates to compounds having the formula



wherein:

- (a) R₁ and R₂ are each independently C₁₋₈ alkyl, C₃₋₇ cycloalkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, C₉₋₁₄ cycloalkylaryl, C₇₋₁₄ alkoxyaryl, C₉₋₁₄ cycloalkoxyaryl, 5-7 membered heteroaryl or C₇₋₁₄ alkylheteroaryl; or R₁ and R₂, together with the nitrogen atom to which they are attached, join to form a 4-8 membered monocyclic heterocycle;
- (b) m is 1 or 2;
- (c) n is 1 or 2;
- (d) R₃ is H or C₁₋₆ alkyl, C₂₋₆ alkenyl or C₃₋₇ cycloalkyl, each optionally substituted with halogen;
- (e) R₄ is C₁₋₈ alkyl optionally substituted with halo, cyano, amino, C₁₋₆ dialkylamino, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, C₁₋₆ alkoxy, carboxy, hydroxy, aryloxy, C₇₋₁₄ alkylaryloxy, C₂₋₆ alkylester or C₈₋₁₅ alkylarylester; C₃₋₁₂ alkenyl; C₃₋₇ cycloalkyl or C₄₋₁₀ alkylcycloalkyl, wherein the cycloalkyl or alkylcycloalkyl are optionally substituted with hydroxy, C₁₋₆ alkyl, C₂₋₆ alkenyl or C₁₋₆ alkoxy; or R₃ together with the carbon atom to which it is attached forms a C₃₋₇ cycloalkyl group optionally substituted with C₂₋₆ alkenyl;

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- (f) Y is H, phenyl substituted with nitro, pyridyl substituted with nitro, or C₁₋₆ alkyl optionally substituted with cyano, hydroxyl or C₃₋₇ cycloalkyl; provided that if R₅ or R₆ is H then Y is H;
- (g) B is H, C₁₋₆ alkyl, R₅-(C=O)-, R₅O(C=O)-, R₅-N(R₆)-C(=O)-, R₅-N(R₆)-C(=S)-, R₅SO₂-, or R₅-N(R₆)-SO₂-;
- (h) R₅ is (i) C₁₋₁₀ alkyl optionally substituted with phenyl, carboxyl, C₁₋₆ alkanoyl, 1-3 halogen, hydroxy, -OC(O)C₁₋₆ alkyl, C₁₋₆ alkoxy, amino optionally substituted with C₁₋₆ alkyl, amido, or (lower alkyl) amido; (ii) C₃₋₇ cycloalkyl, C₃₋₇ cycloalkoxy, or C₄₋₁₀ alkylcycloalkyl, each optionally substituted with hydroxy, carboxyl, (C₁₋₆ alkoxy)carbonyl, amino optionally substituted with C₁₋₆ alkyl, amido, or (lower alkyl) amido; (iii) C₆₋₁₀ aryl or C₇₋₁₆ arylalkyl, each optionally substituted with C₁₋₆ alkyl, halogen, nitro, hydroxy, amido, (lower alkyl) amido, or amino optionally substituted with C₁₋₆ alkyl; (iv) Het; (v) bicyclo(1.1.1)pentane; or (vi) -C(O)OC₁₋₆ alkyl, C₂₋₆alkenyl or C₂₋₆alkynyl;
- (i) R₆ is H; C₁₋₆ alkyl optionally substituted with 1-3 halogens; or C₁₋₆ alkoxy provided R₅ is C₁₋₁₀ alkyl;
- (j) X is O, S, SO, SO₂, OCH₂, CH₂O or NH;
- (k) R' is Het, C₆₋₁₀ aryl or C₇₋₁₄ alkylaryl, each optionally substituted with R^a; and
- (l) R^a is C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₁₋₆ alkoxy, C₃₋₇ cycloalkoxy, halo-C₁₋₆ alkyl, CF₃, mono- or di- halo-C₁₋₆ alkoxy, cyano, halo, thioalkyl, hydroxy, alkanoyl, NO₂, SH, , amino, C₁₋₆ alkylamino, di (C₁₋₆) alkylamino, di (C₁₋₆) alkylamide, carboxyl, (C₁₋₆) carboxyester, C₁₋₆ alkylsulfone, C₁₋₆ alkylsulfonamide, di (C₁₋₆) alkyl(alkoxy)amine, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, or a 5-7 membered monocyclic heterocycle;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

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The present invention also provides compositions comprising the compounds or pharmaceutically acceptable salts, solvates or prodrugs thereof and a pharmaceutically acceptable carrier. In particular, the present invention provides

pharmaceutical compositions useful for inhibiting HCV NS3 comprising a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt, solvate or prodrug thereof, and a pharmaceutically acceptable carrier.

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The present invention further provides methods for treating patients infected with HCV, comprising administering to the patient a therapeutically effective amount of a compound of the present invention, or a pharmaceutically acceptable salt, solvate or prodrug thereof. Additionally, the present invention provides methods of
10 inhibiting HCV NS3 protease by contacting the NS3 protease with a compound of the present invention.

By virtue of the present invention, it is now possible to provide improved drugs comprising the compounds of the invention which can be effective in the
15 treatment of patients infected with HCV. Specifically, the present invention provides peptide compounds that can inhibit the functioning of the NS3 protease, e.g., in combination with the NS4A protease. Further, the present invention makes it possible to administer combination therapy to a patient whereby a compound in accordance with the present invention, which is effective to inhibit the HCV NS3
20 protease, can be administered with another compound having anti-HCV activity, e.g., a compound which is effective to inhibit the function of a target selected from the group consisting of HCV metalloprotease, HCV serine protease, HCV polymerase, HCV helicase, HCV NS4B protein, HCV entry, HCV assembly, HCV egress, HCV NS5A protein, IMPDH and a nucleoside analog for the treatment of an HCV
25 infection.

DETAILED DESCRIPTION OF THE INVENTION

Stereochemical definitions and conventions used herein generally follow
30 McGraw-Hill Dictionary of Chemical Terms, S. P. Parker, Ed., McGraw-Hill Book Company, New York (1984) and Stereochemistry of Organic Compounds, Eliel, E. and Wilen, S., John Wiley & Sons, Inc., New York (1994). Many organic compounds exist in optically active forms, i.e., they have the ability to rotate the

plane of plane-polarized light. In describing an optically active compound, the prefixes D and L or R and S are used to denote the absolute configuration of the molecule about its chiral center(s). The prefixes d and l or (+) and (-) are employed to designate the sign of rotation of plane-polarized light by the compound, with (-) or
5 l meaning that the compound is levorotatory and (+) or d, meaning the compound, is dextrorotatory. For a given chemical structure, these compounds, called stereoisomers, are identical except that they are mirror images of one another. A specific stereoisomer of a mirror image pair may also be referred to as an enantiomer, and a mixture of such isomers is often called an enantiomeric mixture. With
10 reference to the instances where (R) or (S) is used, it is to designate the absolute configuration of a substituent in context to the whole compound and not in context to the substituent alone.

Unless otherwise specifically noted herein, the terms set forth below will have
15 the following definitions.

The terms "racemic mixture" and "racemate" refer to an equimolar mixture of two enantiomeric species, devoid of optical activity.

20 The term "chiral" refers to molecules which have the property of non-superimposability of the mirror image partner, while the term "achiral" refers to molecules which are superimposable on their mirror image partner.

The term "stereoisomers" refers to compounds which have identical chemical
25 composition, but differ with regard to the arrangement of the atoms or groups in space.

The term "diastereomer" refers to a stereoisomer which is not an enantiomer, e.g., a stereoisomer with two or more centers of chirality and whose molecules are
30 not mirror images of one another. Diastereomers have different physical properties, e.g. melting points, boiling points, spectral properties, and reactivities. Mixtures of diastereomers may separate under high resolution analytical procedures such as electrophoresis and chromatography.

The term "enantiomers" refers to two stereoisomers of a compound which are non-superimposable mirror images of one another.

5 The term "pharmaceutically acceptable salt" is intended to include nontoxic salts synthesized from a compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two;
10 generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing Company, Easton, PA, 1990, p. 1445. The compounds of the present invention are useful in the form of the free base or acid or in the form of a pharmaceutically acceptable salt thereof. All forms are
15 within the scope of the invention.

 The term "therapeutically effective amount" means the total amount of each active component that is sufficient to show a meaningful patient benefit, e.g., a sustained reduction in viral load. When applied to an individual active ingredient,
20 administered alone, the term refers to that ingredient alone. When applied to a combination, the term refers to combined amounts of the active ingredients that result in the therapeutic effect, whether administered in combination, serially or simultaneously.

25 The term "compounds of the invention", and equivalent expressions, are meant to embrace compounds of formula I, and pharmaceutically acceptable enantiomer, diastereomer salts, and solvates, e.g. hydrates, and prodrugs. Similarly, references to intermediates, are meant to embrace their salts, and solvates, where the context so permits. References to the compound of the invention also include the
30 preferred compounds, e.g. formula II and A-M.

 The term "derivative" means a chemically modified compound wherein the modification is considered routine by the ordinary skilled chemist, such as an ester or

an amide of an acid, protecting groups, such as a benzyl group for an alcohol or thiol, and tert-butoxycarbonyl group for an amine.

The term "solvate" means a physical association of a compound of this invention with one or more solvent molecules, whether organic or inorganic. This physical association includes hydrogen bonding. In certain instances the solvate will be capable of isolation, for example when one or more solvent molecules are incorporated in the crystal lattice of the crystalline solid. "Solvate" encompasses both solution-phase and isolable solvates. Exemplary solvates include hydrates, ethanولات, methanولات, isopropanولات and the like.

The term "prodrug" as used herein means derivatives of the compounds of the invention which have chemically or metabolically cleavable groups and become, by solvolysis or under physiological conditions, the compounds of the invention which are pharmaceutically active in vivo. A prodrug of a compound may be formed in a conventional manner with a functional group of the compounds such as with an amino, hydroxy or carboxy group when present. The prodrug derivative form often offers advantages of solubility, tissue compatibility, or delayed release in a mammalian organism (see, Bundgard, H., Design of Prodrugs, pp. 7-9, 21-24, Elsevier, Amsterdam 1985). Prodrugs include acid derivatives well known to practitioners of the art, such as, for example, esters prepared by reaction of the parent acidic compound with a suitable alcohol, or amides prepared by reaction of the parent acid compound with a suitable amine.

The term "patient" includes both human and other mammals.

The term "pharmaceutical composition" means a composition comprising a compound of the invention in combination with at least one additional pharmaceutical carrier, i.e., adjuvant, excipient or vehicle, such as diluents, preserving agents, fillers, flow regulating agents, disintegrating agents, wetting agents, emulsifying agents, suspending agents, sweetening agents, flavoring agents, perfuming agents, antibacterial agents, antifungal agents, lubricating agents and dispensing agents, depending on the nature of the mode of administration and dosage

forms. Ingredients listed in Remington's Pharmaceutical Sciences, 18th ed., Mack Publishing Company, Easton, PA (1999) for example, may be used.

5 The phrase "pharmaceutically acceptable" is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of patients without excessive toxicity, irritation, allergic response, or other problem or complication commensurate with a reasonable risk/benefit ratio.

10 The term "treating" refers to: (i) preventing a disease, disorder or condition from occurring in a patient which may be predisposed to the disease, disorder and/or condition but has not yet been diagnosed as having it; (ii) inhibiting the disease, disorder or condition, i.e., arresting its development; and (iii) relieving the disease, disorder or condition, i.e., causing regression of the disease, disorder and/or
15 condition.

 The term "substituted" as used herein includes substitution at from one to the maximum number of possible binding sites on the core, e.p., organic radical, to which the substituent is bonded, e.g., mono-, di-, tri- or tetra- substituted, unless
20 otherwise specifically stated.

 The nomenclature used to describe organic radicals, e.g., hydrocarbons and substituted hydrocarbons, generally follows standard nomenclature known in the art, unless otherwise specifically defined. Combinations of groups, e.g.,
25 alkylalkoxyamine or arylalkyl, include all possible stable configurations, unless otherwise specifically stated. Certain radicals and combinations are defined below for purposes of illustration.

 The term "halo" as used herein means a halogen substituent selected from
30 bromo, chloro, fluoro or iodo. The term "haloalkyl" means an alkyl group that is substituted with one or more halo substituents.

The term "alkyl" as used herein means acyclic, straight or branched chain alkyl substituents having the specified number of carbon atoms and includes, for example, methyl, ethyl, propyl, butyl, tert-butyl, hexyl, 1-methylethyl, 1-methylpropyl, 2-methylpropyl, 1,1-dimethylethyl. Thus, C₁₋₆ alkyl refers to an alkyl group having from one to six carbon atoms. The term "lower alkyl" means an alkyl group having from one to six, preferably from one to four carbon atoms. The term "alkylester" means an alkyl group additionally containing an ester group. Generally, a stated carbon number range, e.g., C₂₋₆ alkylester, includes all of the carbon atoms in the radical.

The term "alkenyl" as used herein means an alkyl radical containing at least one double bond, e.g., ethenyl (vinyl) and alkyl.

The term "alkoxy" as used herein means an alkyl group with the indicated number of carbon atoms attached to an oxygen atom. Alkoxy includes, for example, methoxy, ethoxy, propoxy, 1-methylethoxy, butoxy and 1,1-dimethylethoxy. The latter radical is referred to in the art as tert-butoxy. The term "alkoxycarbonyl" means an alkoxy group additionally containing a carbonyl group.

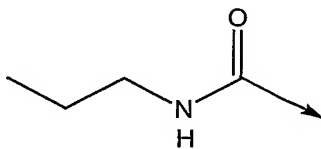
The term "cycloalkyl" as used herein means a cycloalkyl substituent containing the indicated number of carbon atoms and includes, for example, cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and spiro cyclic groups such as spirocyclopropyl as spirocyclobutyl. The term "cycloalkoxy" as used herein means a cycloalkyl group linked to an oxygen atom, such as, for example, cyclobutyloxy or cyclopropyloxy. The term "alkylcycloalkyl" means a cycloalkyl group linked to an alkyl group. The stated carbon number range includes the total number of carbons in the radical, unless otherwise specifically stated. This a C₄₋₁₀ alkylcycloalkyl may contain from 1-7 carbon atoms in the alkyl group and from 3-9 carbon atoms in the ring, e.g., cyclopropylmethyl or cyclohexylethyl.

The term "aryl" as used herein means an aromatic moiety containing the indicated number of carbon atoms, such as, but not limited to phenyl, indanyl or naphthyl. For example, C₆₋₁₀ aryl refers to an aromatic moiety having from six to ten

carbon atoms which may be in the form of a monocyclic or bicyclic structure. The term "haloaryl" as used herein refers to an aryl mono, di or tri substituted with one or more halogen atoms. The terms "alkylaryl", "arylalkyl" and "aralalkyl" mean an aryl group substituted with one or more alkyl groups. Unless the carbon range of each group is specified, the stated range applies to the entire substituent. Thus, a C₇₋₁₄ alkylaryl group may have from 1-8 carbon atoms in the alkyl group for a monocyclic aromatic and from 1-4 carbon atoms in the alkyl group for a fused aromatic. The attachment of the group to bonding site on the molecule can be either at the aryl group or the alkyl group. Unless a specific aryl radical is specified, e.g., fluoro-phenyl, or the radical is stated to be unsubstituted, the aryl radicals include those substituted with typical substituents known to those skilled in the art, e.g., halogen, hydroxy, carboxy, carbonyl, nitro, sulfo, amino, cyano, dialkylamino haloalkyl, CF₃, haloalkoxy, thioalkyl, alkanoyl, SH, alkylamino, alkylamide, dialkylamide, carboxyester, alkylsulfone, alkylsulfonamide and alkyl(alkoxy)amine. Examples of alkylaryl groups include benzyl, butylphenyl and 1-naphthylmethyl.

The term "alkanoyl" as used herein means straight or branched 1-oxoalkyl radicals containing the indicated number of carbon atoms and includes, for example, formyl, acetyl, 1-oxopropyl (propionyl), 2-methyl-1-oxopropyl, 1-oxohexyl and the like.

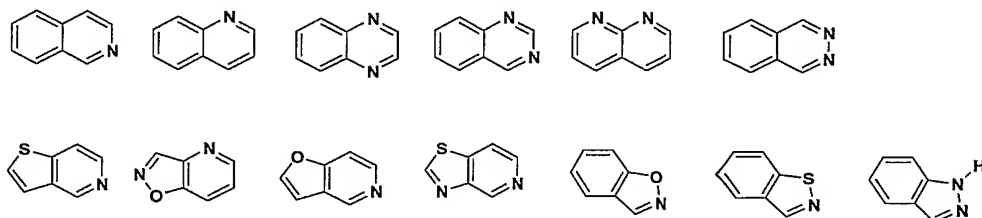
The term "alkylamide" as used herein means an amide mono-substituted with an alkyl, such as



The term "heterocycle", also referred to as "Het", as used herein means 7-12 membered bicyclic heterocycles and 5-7 membered monocyclic heterocycles.

Preferred bicyclic heterocycles are 7-12 membered fused bicyclic ring systems (both rings share an adjacent pair of atoms) containing from one to four heteroatoms selected from nitrogen, oxygen and sulfur, wherein both rings of the heterocycle are fully unsaturated. The nitrogen and sulfur heteroatoms atoms may be optionally oxidized. The bicyclic heterocycle may contain the heteroatoms in one or both rings. Unless a specific heterocycle is specified, e.g., a fluorinated 7-12 membered bicyclic heterocycle, or the heterocycle is stated to be unsubstituted, the heterocycles include those substituted with typical substituents known to those skilled in the art. For example, the bicyclic heterocycle may also contain substituents on any of the ring carbon atoms, e.g., one to three substituents. Examples of suitable substituents include C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₁₋₆ alkoxy, C₃₋₇ cycloalkoxy, halo-C₁₋₆ alkyl, CF₃, mono- or di- halo-C₁₋₆ alkoxy, cyano, halo, thioalkyl, hydroxy, alkanoyl, NO₂, SH, , amino, C₁₋₆ alkylamino, di (C₁₋₆) alkylamino, di (C₁₋₆) alkylamide, carboxyl, (C₁₋₆) carboxyester, C₁₋₆ alkylsulfone, C₁₋₆ alkylsulfonamide, C₁₋₆ alkylsulfoxide, di (C₁₋₆) alkyl(alkoxy)amine, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, and a 5-7 membered monocyclic heterocycle. When two substituents are attached to vicinal carbon atoms of the bicyclic heterocycle, they can join to form a ring, e.g., a five, six or seven membered ring system containing up to two heteroatoms selecting from oxygen and nitrogen. The bicyclic heterocycle may be attached to the molecule, e.g. R₁ in formula I, at any atom in the ring and preferably carbon.

Examples of bicyclic heterocycles include, but are not limited to, the following ring systems:

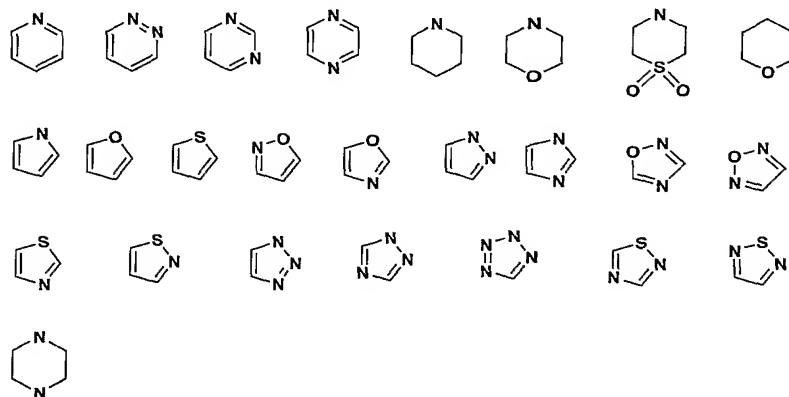


Preferred monocyclic heterocycles are 5-7 membered saturated, partially saturated or fully unsaturated ring system (this latter subset also herein referred to as unsaturated heteroaromatic) containing in the ring from one to four heteroatoms

selected from nitrogen, oxygen and sulfur, wherein the sulfur and nitrogen heteroatoms may be optionally oxidized. Unless a specific heterocycle is specified, e.g., a C₁₋₆ alkoxy substituted 5-7 membered monocyclic heterocycle, or the heterocycle is stated to be unsubstituted, the heterocycles include those substituted with typical substituents known to those skilled in the art. For example, the monocyclic heterocycle may also contain substituents on any of the ring atoms, e.g., one to three substituents. Examples of suitable substituents include C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₁₋₆ alkoxy, C₃₋₇ cycloalkoxy, halo-C₁₋₆ alkyl, CF₃, mono-or di- halo-C₁₋₆ alkoxy, cyano, halo, thioalkyl, hydroxy, alkanoyl, NO₂, SH, , amino, C₁₋₆ alkylamino, di (C₁₋₆) alkylamino, di (C₁₋₆) alkylamide, carboxyl, (C₁₋₆) carboxyester, C₁₋₆ alkylsulfone, C₁₋₆ alkylsulfoxide, C₁₋₆ alkylsulfonamide, di (C₁₋₆) alkyl(alkoxy)amine, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl and an additional 5-7 membered monocyclic heterocycle. The monocyclic heterocycle may be attached to the molecule, e.g. R₁ in formula I, at any atom in the ring.

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Examples of monocyclic heterocycles include, but are not limited to, the following (and their tautomers):



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Those skilled in the art will recognize that the heterocycles used in the compounds of the present invention should be stable. Generally, stable compounds are those which can be synthesized, isolated and formulated using techniques known to those skilled in the art without degradation of the compound.

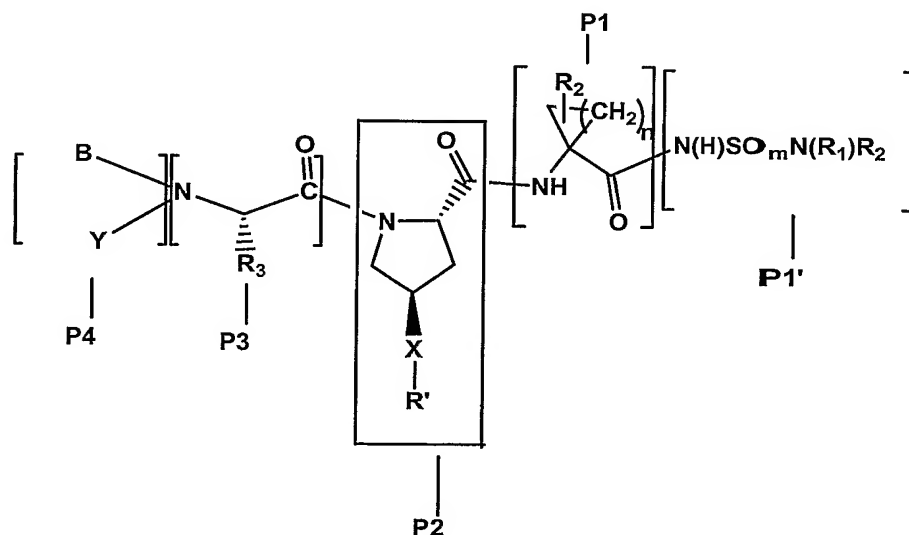
25

The term "substituent" with reference to an amino acid or amino acid derivative means a radical derived from the corresponding α -amino acid. For

instance, the substituents methyl, iso-propyl, and phenyl represent the amino acids alanine, valine, and phenyl glycine, respectively.

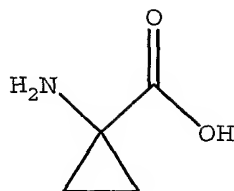
Where used in naming compounds of the present invention, the designations "P1', P1, P2, P3 and P4", as used herein, map the relative positions of the amino acid residues of a protease inhibitor binding relative to the binding of the natural peptide cleavage substrate. Cleavage occurs in the natural substrate between P1 and P1' where the nonprime positions designate amino acids starting from the C-terminus end of the peptide natural cleavage site extending towards the N-terminus; whereas, the prime positions emanate from the N-terminus end of the cleavage site designation and extend towards the C-terminus. For example, P1' refers to the first position away from the right hand end of the C-terminus of the cleavage site (ie. N-terminus first position); whereas P1 starts the numbering from the left hand side of the C-terminus cleavage site, P2: second position from the C-terminus, etc.)(see Berger A. & Schechter I., Transactions of the Royal Society London series (1970), B257, 249-264].

Thus in the compounds of formula I, the "P1' to P4" portions of the molecule are indicated below:

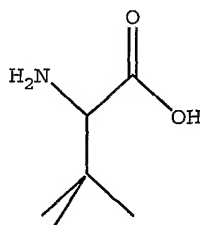


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As used herein the term "1-aminocyclopropyl-carboxylic acid" (Acca) refers to a compound of formula:



As used herein the term "*tert*-butylglycine" refers to a compound of the formula:



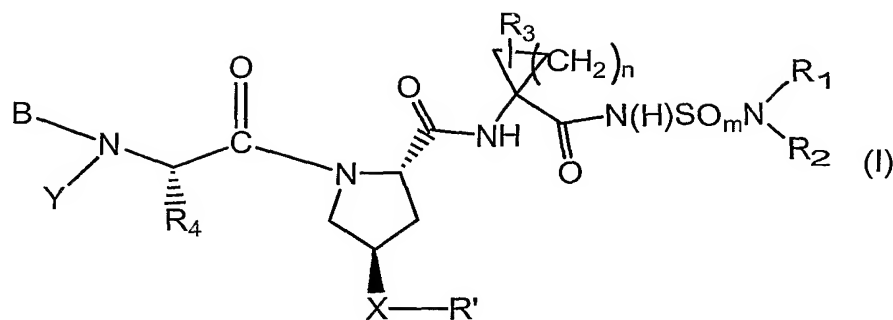
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The term "residue" with reference to an amino acid or amino acid derivative means a radical derived from the corresponding α -amino acid by eliminating the hydroxyl of the carboxy group and one hydrogen of the α -amino acid group. For instance, the terms Gln, Ala, Gly, Ile, Arg, Asp, Phe, Ser, Leu, Cys, Asn, Sar and Tyr represent the "residues" of *L*-glutamine, *L*-alanine, glycine, *L*-isoleucine, *L*-arginine, *L*-aspartic acid, *L*-phenylalanine, *L*-serine, *L*-leucine, *L*-cysteine, *L*-asparagine, sarcosine and *L*-tyrosine, respectively.

The term "side chain" with reference to an amino acid or amino acid residue means a group attached to the α -carbon atom of the α -amino acid. For example, the R-group side chain for glycine is hydrogen, for alanine it is methyl, for valine it is isopropyl. For the specific R-groups or side chains of the α -amino acids reference is made to A.L. Lehninger's text on Biochemistry (see chapter 4).

20

The compounds of the present invention have the structure of Formula I:



- (a) R₁ and R₂ are each independently C₁₋₈ alkyl, C₃₋₇ cycloalkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, C₉₋₁₄ cycloalkylaryl, C₇₋₁₄ alkoxyaryl, C₉₋₁₄ cycloalkoxyaryl, 5-7 membered heteroaryl or C₇₋₁₄ alkylheteroaryl; or R₁ and R₂, together with the nitrogen atom to which they are attached, join to form a 4-8 membered monocyclic heterocycle;
- (b) m is 1 or 2;
- (c) n is 1 or 2;
- (d) R₃ is H; or C₁₋₆ alkyl, C₂₋₆ alkenyl or C₃₋₇ cycloalkyl, each optionally substituted with halogen;
- (e) R₄ is C₁₋₈ alkyl optionally substituted with halo, cyano, amino, C₁₋₆ dialkylamino, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, C₁₋₆ alkoxy, carboxy, hydroxy, aryloxy, C₇₋₁₄ alkylaryloxy, C₂₋₆ alkylester or C₈₋₁₅ alkylarylester; C₃₋₁₂ alkenyl; C₃₋₇ cycloalkyl or C₄₋₁₀ alkylcycloalkyl, wherein the cycloalkyl or alkylcycloalkyl are optionally substituted with hydroxy, C₁₋₆ alkyl, C₂₋₆ alkenyl or C₁₋₆ alkoxy; or R₃ together with the carbon atom to which it is attached forms a C₃₋₇ cycloalkyl group optionally substituted with C₂₋₆ alkenyl;
- (f) Y is H, phenyl substituted with nitro, pyridyl substituted with nitro, or C₁₋₆ alkyl optionally substituted with cyano, hydroxyl or C₃₋₇ cycloalkyl; provided that if R₅ or R₆ is H then Y is H;
- (g) B is H, C₁₋₆ alkyl, R₅-(C=O)-, R₅O(C=O)-, R₅-N(R₆)-C(=O)-, R₅-N(R₆)-C(=S)-, R₅SO₂-, or R₅-N(R₆)-SO₂-;
- (h) R₅ is (i) C₁₋₁₀ alkyl optionally substituted with phenyl, carboxyl, C₁₋₆ alkanoyl, 1-3 halogen, hydroxy, -OC(O)C₁₋₆ alkyl, C₁₋₆ alkoxy, amino

- optionally substituted with C₁₋₆ alkyl, amido, or (lower alkyl) amido;
 (ii) C₃₋₇ cycloalkyl, C₃₋₇ cycloalkoxy, or C₄₋₁₀ alkylcycloalkyl, each
 optionally substituted with hydroxy, carboxyl, (C₁₋₆ alkoxy)carbonyl,
 amino optionally substituted with C₁₋₆ alkyl, amido, or (lower alkyl)
 amido; (iii) C₆₋₁₀ aryl or C₇₋₁₆ arylalkyl, each optionally substituted
 with C₁₋₆ alkyl, halogen, nitro, hydroxy, amido, (lower alkyl) amido,
 or amino optionally substituted with C₁₋₆ alkyl; (iv) Het; (v)
 bicyclo(1.1.1)pentane; or (vi) -C(O)OC₁₋₆ alkyl, C₂₋₆alkenyl or C₂₋₆
 alkynyl;
- (i) R₆ is H; C₁₋₆ alkyl optionally substituted with 1-3 halogens; or C₁₋₆
 alkoxy provided R₅ is C₁₋₁₀ alkyl;
- (j) X is O, S, SO, SO₂, OCH₂, CH₂O or NH;
- (k) R' is Het, C₆₋₁₀ aryl or C₇₋₁₄ alkylaryl, each optionally substituted with
 R^a; and
- (l) R^a is C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₁₋₆ alkoxy, C₃₋₇ cycloalkoxy, halo-C₁₋₆
 alkyl, CF₃, mono- or di- halo-C₁₋₆ alkoxy, cyano, halo, thioalkyl,
 hydroxy, alkanoyl, NO₂, SH, , amino, C₁₋₆ alkylamino, di (C₁₋₆)
 alkylamino, di (C₁₋₆) alkylamide, carboxyl, (C₁₋₆) carboxyester, C₁₋₆
 alkylsulfone, C₁₋₆ alkylsulfonamide, di (C₁₋₆) alkyl(alkoxy)amine, C₆₋₁₀
 aryl, C₇₋₁₄ alkylaryl, or a 5-7 membered monocyclic heterocycle;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Preferably, R₁ and R₂ are each independently C₁₋₈ alkyl, C₃₋₇ cycloalkyl, C₄₋₁₀
 alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, 5-7 membered heteroaryl or
 C₇₋₁₄ alkylheteroaryl; or R₁ and R₂, together with the nitrogen atom to which they are
 attached, join to form a 4-8 membered monocyclic heterocycle. More preferably, R₁
 and R₂ are each independently C₁₋₈ alkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀
 aryl, C₇₋₁₄ alkylaryl, 5-7 membered heteroaryl or C₇₋₁₄ alkylheteroaryl, or R₁ and R₂,
 together with the nitrogen atom to which they are attached, join to form a 5-6
 membered monocyclic heterocycle.

Preferably, R₃ is C₁₋₆ alkyl, C₂₋₆ alkenyl or C₃₋₇ cycloalkyl. More preferably, R₃ is C₂₋₆ alkenyl.

Preferably, R₄ is C₁₋₈ alkyl optionally substituted with C₆ aryl, C₁₋₆ alkoxy, carboxy, hydroxy, aryloxy, C₇₋₁₄ alkylaryloxy, C₂₋₆ alkylester or C₈₋₁₅ alkylarylester; C₃₋₁₂ alkenyl; C₃₋₇ cycloalkyl; or C₄₋₁₀ alkylcycloalkyl. More preferably, R₄ is C₁₋₈ alkyl optionally substituted with C₁₋₆ alkoxy; or C₃₋₇ cycloalkyl.

Preferably, Y is H.

Preferably, B is H, C₁₋₆ alkyl, R₅-(C=O)-, R₅O(C=O)-, R₅-N(R₆)-C(=O)-, R₅-N(R₆)-C(=S)-, R₅SO₂-, or R₅-N(R₆)-SO₂-. More preferably, B is R₅-(C=O)-, R₅O(C=O)-, or R₅-N(R₆)-C(=O)-. Even more preferably, B is R₅O(C=O)- and R₅ is C₁₋₆ alkyl.

Preferably, R₅ is (i) C₁₋₁₀ alkyl optionally substituted with phenyl, carboxyl, C₁₋₆ alkanoyl, 1-3 halogen, hydroxy, C₁₋₆ alkoxy; (ii) C₃₋₇ cycloalkyl, C₃₋₇ cycloalkoxy, or C₄₋₁₀ alkylcycloalkyl; or (iii) C₆₋₁₀ aryl or C₇₋₁₆ arylalkyl, each optionally substituted with C₁₋₆ alkyl or halogen. More preferably, R₅ is (i) C₁₋₁₀ alkyl optionally substituted with 1-3 halogen or C₁₋₆ alkoxy; or (ii) C₃₋₇ cycloalkyl or C₄₋₁₀ alkylcycloalkyl.

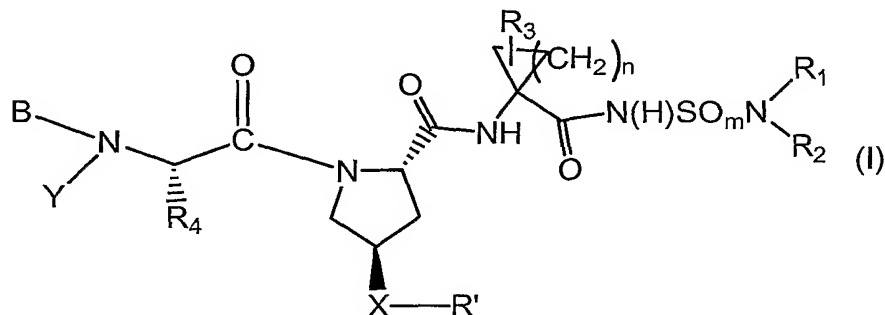
Preferably, R₆ is H or C₁₋₆ alkyl optionally substituted with 1-3 halogens. More preferably, R₆ is H.

Preferably, X is O or NH.

Preferably, R' is Het; or C₆₋₁₀ aryl optionally substituted with R^a. More preferably, R' is Het. Even more preferably, the heterocycle contains 1 or 2 nitrogen atoms and optionally a sulfur atom or an oxygen atom in the ring. Preferably, the heterocycle is substituted with at least one of C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, or a 5-7 membered monocyclic heterocycle.

Preferably, R^a is C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₁₋₆ alkoxy, halo-C₁₋₆ alkyl, halo, amino, C₆ aryl, or a 5-7 membered monocyclic heterocycle.

In a preferred aspect of the invention, there is provided a compound having the
5 formula



wherein:

- 10 (a) R₁ and R₂ are each independently C₁₋₈ alkyl, C₃₋₇ cycloalkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, 5-7 membered heteroaryl, C₇₋₁₄ alkylheteroaryl, or R₁ and R₂, together with the nitrogen atom to which they are attached, join to form a 4-8 membered monocyclic heterocycle;
- 15 (b) m is 1 or 2;
- (c) n is 1 or 2;
- (d) R₃ is C₁₋₆ alkyl, C₂₋₆ alkenyl or C₃₋₇ cycloalkyl;
- (e) R₄ is C₁₋₈ alkyl optionally substituted with C₆ aryl, C₁₋₆ alkoxy, carboxy, hydroxy, aryloxy, C₇₋₁₄ alkylaryloxy, C₂₋₆ alkylester, C₈₋₁₅ alkylarylester; C₃₋₁₂ alkenyl, C₃₋₇ cycloalkyl, or C₄₋₁₀ alkylcycloalkyl;
- 20 (f) B is H, C₁₋₆ alkyl, R₅-(C=O)-, R₅O(C=O)-, R₅-N(R₆)-C(=O)-, R₅-N(R₆)-C(=S)-, R₅SO₂-, or R₅-N(R₆)-SO₂-;
- (g) R₅ is (i) C₁₋₁₀ alkyl optionally substituted with phenyl, carboxyl, C₁₋₆ alkanoyl, 1-3 halogen, hydroxy, C₁₋₆ alkoxy; (ii) C₃₋₇ cycloalkyl, C₃₋₇ cycloalkoxy, or C₄₋₁₀ alkylcycloalkyl; or (iii) C₆₋₁₀ aryl or C₇₋₁₆ arylalkyl, each optionally substituted with C₁₋₆ alkyl or halogen;
- 25

- (h) R_6 is H or C_{1-6} alkyl optionally substituted with 1-3 halogens;
(i) X is O or NH;
(j) R' is Het; or C_{6-10} aryl optionally substituted with R^a ; and
(k) R^a is C_{1-6} alkyl, C_{3-7} cycloalkyl, C_{1-6} alkoxy, halo- C_{1-6} alkyl, halo,
5 amino, C_6 aryl, or a 5-7 membered monocyclic heterocycle;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

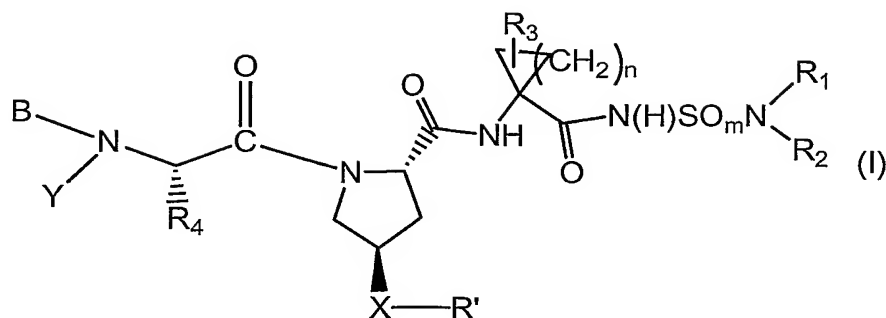
Preferably, R_1 and R_2 are each independently C_{1-8} alkyl, C_{4-10} alkylcycloalkyl,
10 C_{1-6} alkoxy, C_{6-10} aryl, C_{7-14} alkylaryl, 5-7 membered heteroaryl, C_{7-14}
alkylheteroaryl, or R_1 and R_2 , together with the nitrogen atom to which they are
attached, join to form a 5-6 membered monocyclic heterocycle. More preferably, R_1
and R_2 are each independently C_{1-3} alkyl or C_{1-3} alkoxy.

15 Preferably, R' is a bicyclic heterocycle. More preferably, the heterocycle
contains 1 or 2 nitrogen atoms and optionally a sulfur atom or an oxygen atom in the
ring. Preferably, the heterocycle is substituted with at least one of C_{1-6} alkyl, C_{1-6}
alkoxy, halo, C_6 aryl, and a 5-7 membered monocyclic heterocycle. Even more
preferably, R' is a bicyclic heterocycle containing 1 nitrogen atom and substituted
20 with methoxy and at least one of a C_6 aryl and a 5-7 membered monocyclic
heterocycle.

In another preferred aspect, R' is a monocyclic heterocycle. Preferably, the
heterocycle contains 1 or 2 nitrogen atoms and optionally a sulfur atom or an oxygen
25 atom in the ring. More preferably, the heterocycle is substituted with at
least one of C_{1-6} alkyl, C_{1-6} alkoxy, halo, C_{6-10} aryl, C_{7-14} alkylaryl, or a 5-7 membered
monocyclic heterocycle. Preferably, R' is a monocyclic heterocycle containing
1 or 2 nitrogen atoms and substituted with methoxy and at least one of a C_6 aryl and
a 5-7 membered monocyclic heterocycle.

30

In another preferred aspect of the invention, there is provided a compound
having the formula



wherein:

- (a) R₁ and R₂ are each independently C₁₋₈ alkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, 5-7 membered heteroaryl or C₇₋₁₄ alkylheteroaryl; or R₁ and R₂, together with the nitrogen atom to which they are attached, join to form a 5-6 membered monocyclic heterocycle;
- (b) m is 1 or 2;
- (c) n is 1 or 2;
- (d) R₃ is C₂₋₆ alkenyl;
- (e) R₄ is C₁₋₈ alkyl;
- (f) B is R₅O(C=O)-, or R₅-NH-C(=O)-;
- (g) R₅ is C₁₋₁₀ alkyl;
- (h) R' is a bicyclic heterocycle optionally substituted with R^a; and
- (i) R^a is C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, C₆ aryl, or a 5-7 membered monocyclic heterocycle;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

Preferably, R₁ and R₂ are each independently C₁₋₃ alkyl, or C₁₋₃ alkoxy. More preferably, R₁ is methyl. Preferably, R₂ is methyl or methoxy. Preferably, R₃ is vinyl. Preferably, R₄ is t-butyl. Preferably, R₅ is t-butyl. Preferably, R' is quinoline or isoquinoline optionally substituted with R^a.

Preferably, R₁ is methyl, R₂ is methoxy, R₃ is vinyl, R₄ is t-butyl, R₅ is t-butyl, and R' is isoquinoline substituted with at least one R^a. Preferably, R^a is C₁₋₆

alkoxy. More preferably, R^a further includes at least one of C₆ aryl or a 5-7 membered monocyclic heterocycle.

The compounds of the present invention, which contain a basic moiety, can form salts by the addition of a pharmaceutically acceptable acid. The acid addition salts are formed from a compound of Formula I and a pharmaceutically acceptable inorganic acid, including but not limited to hydrochloric, hydrobromic, hydroiodic, sulfuric, phosphoric, or organic acid such as *p*-toluenesulfonic, methanesulfonic, acetic, benzoic, citric, malonic, fumaric, maleic, oxalic, succinic, sulfamic, or tartaric. Thus, examples of such pharmaceutically acceptable salts include chloride, bromide, iodide, sulfate, phosphate, methanesulfonate, citrate, acetate, malonate, fumarate, sulfamate, and tartrate.

Salts of an amine group may also comprise quaternary ammonium salts in which the amino nitrogen carries a suitable organic group such as an alkyl, alkenyl, alkynyl or aralkyl moiety.

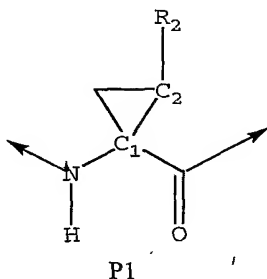
Compounds of the present invention, which are substituted with an acidic group, may exist as salts formed through base addition. Such base addition salts include those derived from inorganic bases which include, for example, alkali metal salts (e.g. sodium and potassium), alkaline earth metal salts (e.g. calcium and magnesium), aluminum salts and ammonium salts. In addition, suitable base addition salts include salts of physiologically acceptable organic bases such as trimethylamine, triethylamine, morpholine, pyridine, piperidine, picoline, dicyclohexylamine, N,N'-dibenzylethylenediamine, 2-hydroxyethylamine, bis-(2-hydroxyethyl)amine, tri-(2-hydroxyethyl)amine, procaine, dibenzylpiperidine, N-benzyl- β -phenethylamine, dehydroabietylamine, N,N'-bishydroabietylamine, glucamine, N-methylglucamine, collidine, quinine, quinoline, ethylenediamine, ornithine, choline, N,N'-benzylphenethylamine, chloroprocaine, diethanolamine, diethylamine, piperazine, tris(hydroxymethyl)aminomethane and tetramethylammonium hydroxide and basic amino acids such as lysine, arginine and N-methylglutamine. These salts may be prepared by methods known to those skilled in the art.

Certain compounds of the present invention, and their salts, may also exist in the form of solvates with water, for example hydrates, or with organic solvents such as methanol, ethanol or acetonitrile to form, respectively, a methanolate, ethanolate or acetonitrilate. The present invention includes each solvate and mixtures thereof.

In addition, compounds of the present invention, or a salt or solvate thereof, may exhibit polymorphism. The present invention also encompasses any such polymorphic form.

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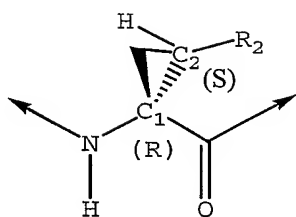
The compounds of the present invention also contain two or more chiral centers. For example, the compounds may include P1 cyclopropyl element of formula



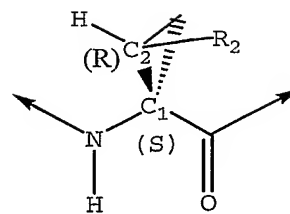
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wherein C₁ and C₂ each represent an asymmetric carbon atom at positions 1 and 2 of the cyclopropyl ring. Notwithstanding other possible asymmetric centers at other segments of the compounds, the presence of these two asymmetric centers means that the compounds can exist as racemic mixtures of diastereomers, such as the diastereomers wherein R₂ is configured either syn to the amide or syn to the carbonyl as shown below.

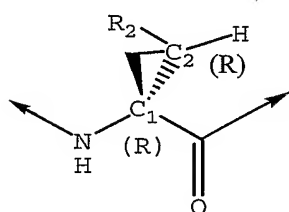
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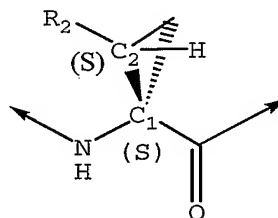
(1R, 2S)

 R_2 is syn to carbonyl

(1S, 2R)

 R_2 is syn to carbonyl

(1R, 2R)

 R_2 is syn to amide

(1S, 2S)

 R_2 is syn to amide

The present invention includes both enantiomers and mixtures of enantiomers such as racemic mixtures.

5

The enantiomers may be resolved by methods known to those skilled in the art, for example, by formation of diastereoisomeric salts which may be separated by crystallization, gas-liquid or liquid chromatography, selective reaction of one enantiomer with an enantiomer-specific reagent. It will be appreciated that where the desired enantiomer is converted into another chemical entity by a separation technique, then an additional step is required to form the desired enantiomeric form. Alternatively, specific enantiomers may be synthesized by asymmetric synthesis using optically active reagents, substrates, catalysts or solvents, or by converting one enantiomer into the other by asymmetric transformation.

15

The compounds of the present invention may be in the form of a prodrug. Simple aliphatic or aromatic esters derived from, when present, acidic groups pendent on the compounds of this invention are preferred prodrugs. In some cases it

is desirable to prepare double ester type prodrugs such as (acyloxy) alkyl esters or (alkoxycarbonyl)oxy)alkyl esters.

5 Certain compounds of the present invention may also exist in different stable conformational forms which may be separable. Torsional asymmetry due to restricted rotation about an asymmetric single bond, for example because of steric hindrance or ring strain, may permit separation of different conformers. The present invention includes each conformational isomer of these compounds and mixtures thereof.

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Certain compounds of the present invention may exist in zwitterionic form and the present invention includes each zwitterionic form of these compounds and mixtures thereof.

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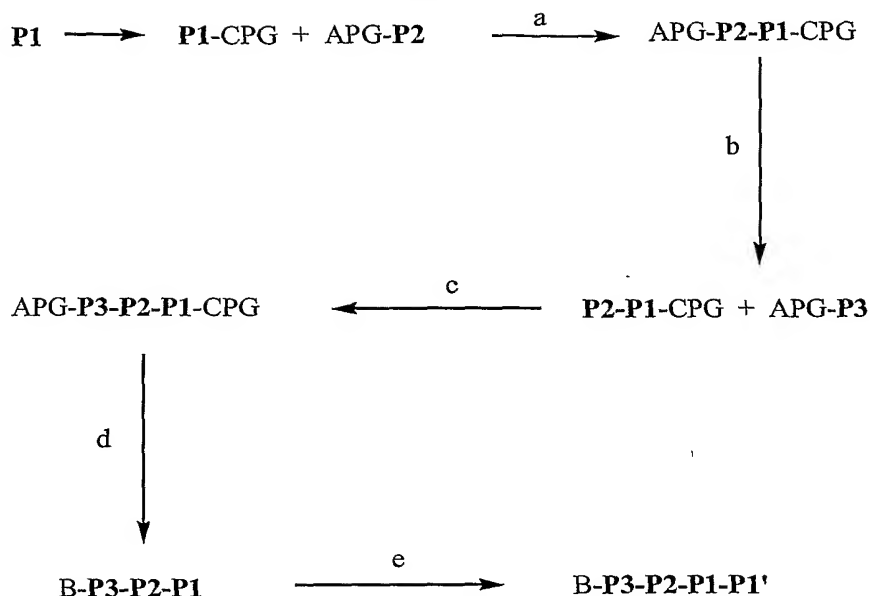
The starting materials useful to synthesize the compounds of the present invention are known to those skilled in the art and can be readily manufactured or are commercially available.

20 The compounds of the present invention can be manufactured by methods known to those skilled in the art, see e.g., US Patent No. 6,323,180 and US Patent Application Publication No. 20020111313 A1, published August 15, 2002. The following methods set forth below are provided for illustrative purposes and are not intended to limit the scope of the claimed invention. It will be recognized that it may be preferred or necessary to prepare such a compound in which a functional group is protected using a conventional protecting group then to remove the protecting group to provide a compound of the present invention. The details concerning the use of protecting groups in accordance with the present invention are known to those skilled in the art.

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The compounds of the present invention may, for example, be synthesized according to a general process as illustrated in Scheme I (wherein CPG is a carboxyl protecting group and APG is an amino protecting group):

Scheme I

Briefly, the P1, P2, and P3 can be linked by well known peptide coupling techniques. The P1, P2, and P3 groups may be linked together in any order as long as the final compound corresponds to peptides of the invention. For example, P3 can be linked to P2-P1; or P1 linked to P3-P2.

Generally, peptides are elongated by deprotecting the α -amino group of the N-terminal residue and coupling the unprotected carboxyl group of the next suitably N-protected amino acid through a peptide linkage using the methods described. This deprotection and coupling procedure is repeated until the desired sequence is obtained. This coupling can be performed with the constituent amino acids in stepwise fashion, as depicted in Scheme I.

Coupling between two amino acids, an amino acid and a peptide, or two peptide fragments can be carried out using standard coupling procedures such as the azide method, mixed carbonic-carboxylic acid anhydride (isobutyl chloroformate) method, carbodiimide (dicyclohexylcarbodiimide, diisopropylcarbodiimide, or water-soluble carbodiimide) method, active ester (*p*-nitrophenyl ester, *N*-hydroxysuccinic imido ester) method, Woodward reagent K-method, carbonyldiimidazole method, phosphorus reagents or oxidation-reduction methods.

Some of these methods (especially the carbodiimide method) can be enhanced by adding 1-hydroxybenzotriazole or 4-DMAP. These coupling reactions can be performed in either solution (liquid phase) or solid phase.

5 More explicitly, the coupling step involves the dehydrative coupling of a free carboxyl of one reactant with the free amino group of the other reactant in the present of a coupling agent to form a linking amide bond. Descriptions of such coupling agents are found in general textbooks on peptide chemistry, for example, M. Bodanszky, "Peptide Chemistry", 2nd rev ed., Springer-Verlag, Berlin, Germany,
10 (1993). Examples of suitable coupling agents are N,N'-dicyclohexylcarbodiimide, 1-hydroxybenzotriazole in the presence of N,N'-dicyclohexylcarbodiimide or N-ethyl-N'-[(3-dimethylamino)propyl]carbodiimide. A practical and useful coupling agent is the commercially available (benzotriazol-1-yloxy)tris-(dimethylamino)phosphonium hexafluorophosphate, either
15 by itself or in the present of 1-hydroxybenzotriazole or 4-DMAP. Another practical and useful coupling agent is commercially available 2-(1H-benzotriazol-1-yl)-N, N, N', N'-tetramethyluronium tetrafluoroborate. Still another practical and useful coupling agent is commercially available O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.
20 The coupling reaction is conducted in an inert solvent, e.g. dichloromethane, acetonitrile or dimethylformamide. An excess of a tertiary amine, e.g. diisopropylethylamine, N-methylmorpholine, N-methylpyrrolidine or 4-DMAP is added to maintain the reaction mixture at a pH of about 8. The reaction temperature usually ranges between 0 °C and 50 °C and the reaction time usually ranges between
25 15 min and 24 h.

The functional groups of the constituent amino acids generally must be protected during the coupling reactions to avoid formation of undesired bonds. Protecting groups that can be used are listed, for example, in Greene, "Protective
30 Groups in Organic Chemistry", John Wiley & Sons, New York (1981) and "The Peptides: Analysis, Synthesis, Biology", Vol. 3, Academic Press, New York (1981), the disclosures of which are hereby incorporated by reference.

The α -amino group of each amino acid to be coupled to the growing peptide chain must be protected (APG). Any protecting group known in the art can be used. Examples of such groups include: 1) acyl groups such as formyl, trifluoroacetyl, phthalyl, and *p*-toluenesulfonyl; 2) aromatic carbamate groups such as benzyloxycarbonyl (Cbz or Z) and substituted benzyloxycarbonyls, and 9-fluorenylmethyloxycarbonyl (Fmoc); 3) aliphatic carbamate groups such as tert-butyloxycarbonyl (Boc), ethoxycarbonyl, diisopropylmethoxycarbonyl, and allyloxycarbonyl; 4) cyclic alkyl carbamate groups such as cyclopentyloxycarbonyl and adamantyloxycarbonyl; 5) alkyl groups such as triphenylmethyl and benzyl; 6)trialkylsilyl such as trimethylsilyl; and 7) thiol containing groups such as phenylthiocarbonyl and dithiasuccinoyl.

The preferred α -amino protecting group is either Boc or Fmoc. Many amino acid derivatives suitably protected for peptide synthesis are commercially available. The α -amino protecting group of the newly added amino acid residue is cleaved prior to the coupling of the next amino acid. When the Boc group is used, the methods of choice are trifluoroacetic acid, neat or in dichloromethane, or HCl in dioxane or in ethyl acetate. The resulting ammonium salt is then neutralized either prior to the coupling or in situ with basic solutions such as aqueous buffers, or tertiary amines in dichloromethane or acetonitrile or dimethylformamide. When the Fmoc group is used, the reagents of choice are piperidine or substituted piperidine in dimethylformamide, but any secondary amine can be used. The deprotection is carried out at a temperature between 0°C and room temperature (rt or RT) usually 20-22°C.

Any of the amino acids having side chain functionalities must be protected during the preparation of the peptide using any of the above-described groups. Those skilled in the art will appreciate that the selection and use of appropriate protecting groups for these side chain functionalities depend upon the amino acid and presence of other protecting groups in the peptide. The selection of such protecting groups is important in that the group must not be removed during the deprotection and coupling of the α -amino group.

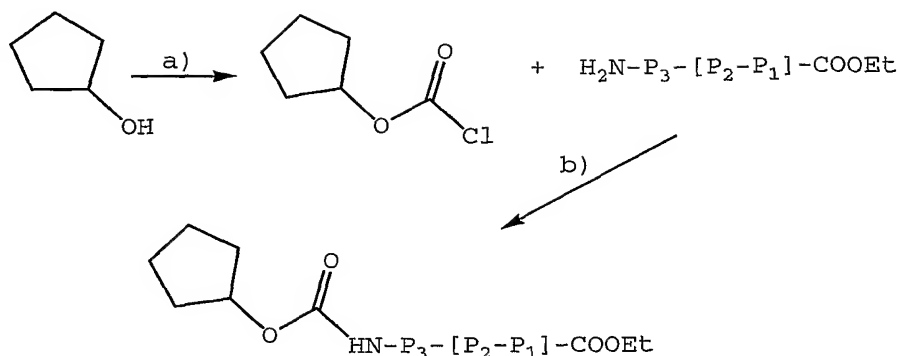
For example, when Boc is used as the α -amino protecting group, the following side chain protecting group are suitable: *p*-toluenesulfonyl (tosyl) moieties can be used to protect the amino side chain of amino acids such as Lys and Arg; acetamidomethyl, benzyl (Bn), or *tert*-butylsulfonyl moieties can be used to protect the sulfide containing side chain of cysteine; benzyl (Bn) ethers can be used to protect the hydroxy containing side chains of serine, threonine or hydroxyproline; and benzyl esters can be used to protect the carboxy containing side chains of aspartic acid and glutamic acid.

When Fmoc is chosen for the α -amine protection, usually *tert*-butyl based protecting groups are acceptable. For instance, Boc can be used for lysine and arginine, *tert*-butyl ether for serine, threonine and hydroxyproline, and *tert*-butyl ester for aspartic acid and glutamic acid. Triphenylmethyl (Trityl) moiety can be used to protect the sulfide containing side chain of cysteine.

Once the elongation of the peptide is completed all of the protecting groups are removed. When a liquid phase synthesis is used, the protecting groups are removed in whatever manner is dictated by the choice of protecting groups. These procedures are well known to those skilled in the art.

Further, the following guidance may be followed in the preparation of compounds of the present invention. For example, to form a compound where $R_4-C(O)-$, $R_4-S(O)_2-$, a protected P3 or the whole peptide or a peptide segment is coupled to an appropriate acyl chloride or sulfonyl chloride respectively, that is either commercially available or for which the synthesis is well known in the art. In preparing a compound where $R_4O-C(O)-$, a protected P3 or the whole peptide or a peptide segment is coupled to an appropriate chloroformate that is either commercially available or for which the synthesis is well known in the art. For Boc-derivatives $(Boc)_2O$ is used.

For example:



Cyclopentanol is treated with phosgene to furnish the corresponding chloroformate.

5

The chloroformate is treated with the desired NH_2 -tripeptide in the presence of a base such as triethylamine to afford the cyclopentylcarbamate.

In preparing a compound where $\text{R}_4-\text{N}(\text{R}_5)-\text{C}(\text{O})-$, or $\text{R}_4-\text{NH}-\text{C}(\text{S})-$, a protected P3 or the whole peptide or a peptide segment is treated with phosgene followed by amine as described in SynLett. Feb 1995; (2); 142-144 or is reacted with the commercially available isocyanate and a suitable base such as triethylamine.

In preparing a compound where $\text{R}_4-\text{N}(\text{R}_5)-\text{S}(\text{O}_2)$, a protected P3 or the whole peptide or a peptide segment is treated with either a freshly prepared or commercially available sulfamyl chloride followed by amine as described in patent Ger. Offen.(1998), 84 pp. DE 19802350 or WO 98/32748.

The α -carboxyl group of the C-terminal residue is usually protected as an ester (CPG) that can be cleaved to give the carboxylic acid. Protecting groups that can be used include: 1) alkyl esters such as methyl, trimethylsilylethyl and t-butyl, 2) aralkyl esters such as benzyl and substituted benzyl, or 3) esters that can be cleaved by mild base treatment or mild reductive means such as trichloroethyl and phenacyl esters.

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The resulting α -carboxylic acid (resulting from cleavage by mild acid, mild base treatment or mild reductive means) is coupled with a $R_1(R_2)NSO_2NH_2$ [prepared by described herein] in the presence of peptide coupling agent such as CDI in the presence of a base such as LiHMDS (lithium bis-hexamethyldisilylamide) to

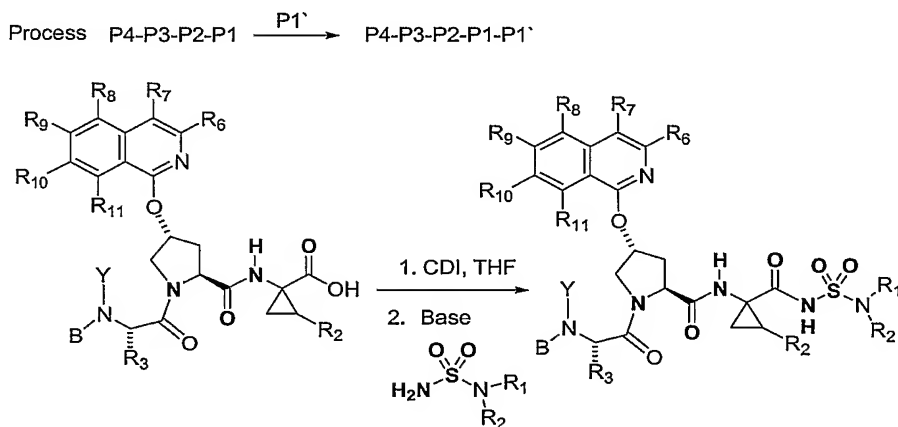
5 incorporate the P1' moiety, effectively assembling the tripeptide P1'-P1-P2-P3-APG.

Scheme II further shows the general process wherein compounds of Formula I are constructed by the coupling of tripeptide carboxylic acid intermediate (1) with a P1' sulfamide. (It should be noted that the groups $R_6, R_7, R_8, R_9, R_{10}, R_{11}$ as shown

10 below represent substituents of the heterocyclic system.) Said coupling reaction requires treatment of carboxylic acid (1) with a coupling reagent such as carbonyl diimidazole in a solvent such as THF, which can be heated to reflux, followed by the addition of the formed derivative of (1), to the P1' sulfamide, in a solvent such as THF or methylene chloride in the presence of a base such as lithium

15 bis-hexamethyldisilylamide.

Scheme II



An alternative process for the construction of compounds of Formula I is

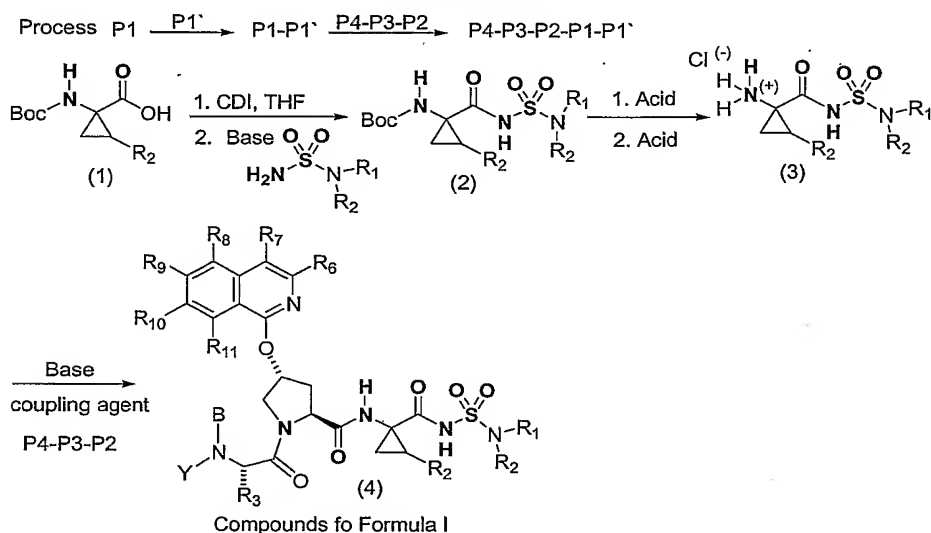
20 shown in Scheme III. Therein the P1' sulfamide element is coupled to the P1 element using the process employed in Scheme II. The resulting P1-P1' moiety can then be deprotected at its amino terminus. In this general example a Boc protecting group is employed but one skilled in the art would recognize that a number of suitable amino protecting groups could be employed in this process. Said Boc

25 protecting group can be removed using acid such as trifluoroacetic acid in a solvent

such as dichloroethane to provide the deprotected amine as the TFA salt. Said TFA amine salt can be directly employed in the subsequent coupling reaction or as an alternative the TFA amine salt can be first converted to the HCl amine salt, and this HCl amine salt is used in said coupling reaction as shown in Scheme III. The

5 coupling of said HCl amine salt (3) with the carboxyl terminus a P4-P3-P2 intermediate can be achieved using coupling reagents, such as HATU, in solvents such as dichloromethane to provide compounds of Formula I (4).

Scheme III

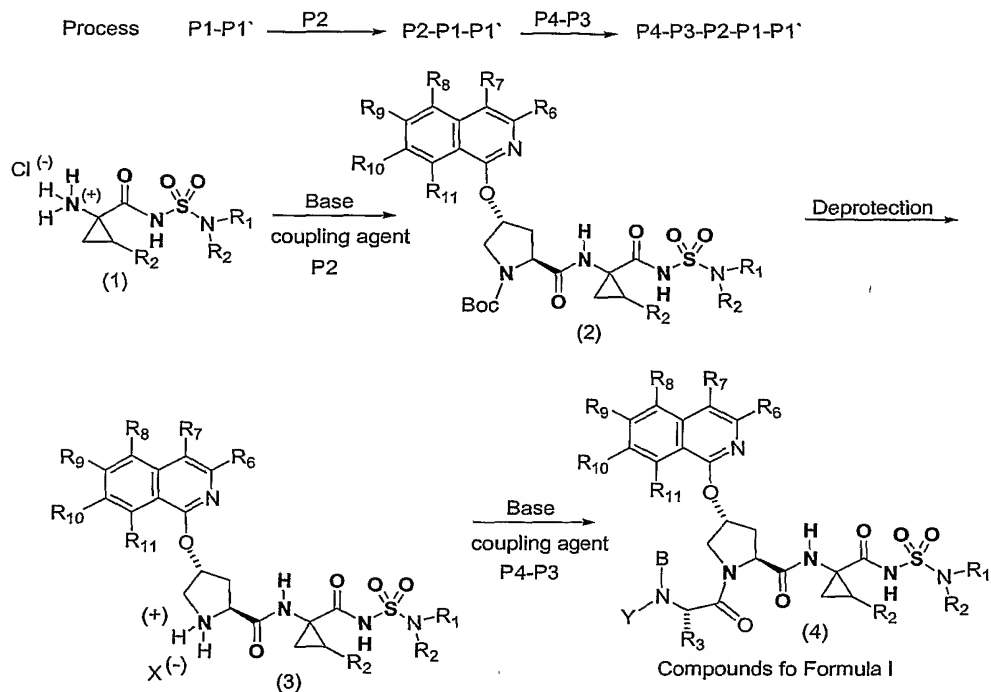


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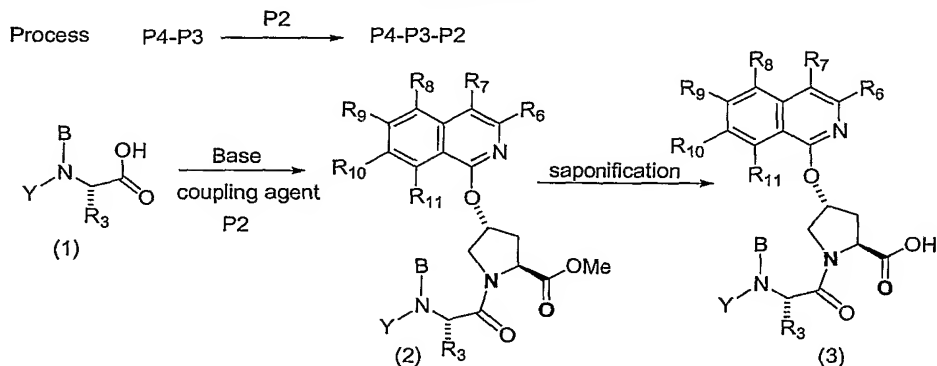
An alternative process for the construction of compounds of Formula I is shown in Scheme IV. Herein the hydrochloride salt of the P1-P1' terminal amine (1) is coupled to the free carboxyl group of the P2 element using coupling agents such as PyBOP, in the presence of a base such as diisopropyl amine, and in a solvent such as methylene chloride. The resulting P2-P1-P1' intermediate can be converted to compounds of Formula I in a two step process wherein the first step is deprotection of the P2 amine terminus using an acid such as TFA in a solvent such as methylene chloride. The resulting trifluoroacetic acid salt can be coupled with the carboxyl terminus of the P4-P3 element using standard coupling agents such as PyBop in the presence of base such as diisopropyl amine, and using solvents such methylene chloride to provide compounds of Formula I (4).

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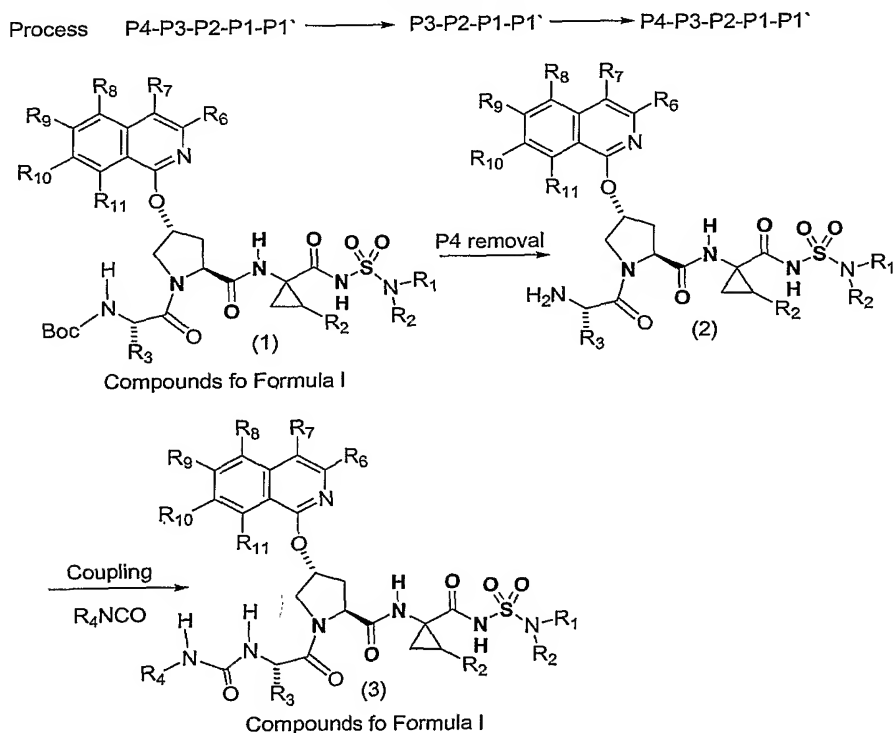
Scheme IV

- The P4-P3-P2 intermediate utilized in the above schemes can be constructed as previously described with a further description of this process shown in general Scheme V. Therein the free carboxyl terminus of the P4-P3 intermediate (1), can be coupled to the amino terminus of the P2 element to provide the P4-P3-P2 dipeptide (2). The carboxyl terminus of the P4-P3-P2 intermediate can be deprotected by saponification of the ester group to provide P4-P3-P2 as the free carboxylic acid (3).
- Intermediates like (3) can be converted to compounds of Formula I using the methods described herein.

Scheme V

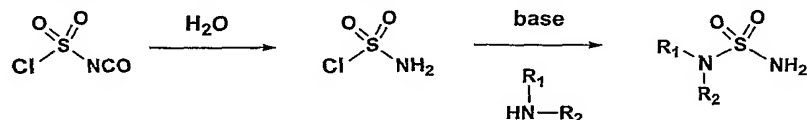
Compounds of Formula 1 can also be converted into other compounds of Formula I as described herein. An example of such a process is shown in Scheme VI wherein a compound of Formula I (1) which bears a Boc group at the P4 position is converted to a compound of Formula I (3) wherein said compound bears a urea group at the P4 position. The conversion of (1) to (3) can be carried out in a two step process the first of which is the conversion of (1) to amine (2) by treatment of (1) with an acid such as TFA in a solvent such as methylene chloride. The resulting amine TFA salt can be treated with an isocyanate in the presence of one equivalent of base to provide a compound of Formula I (3) wherein the P3 moiety is capped with a urea. As previously noted one skilled in the art will recognize that intermediate (2) can be used as starting materials for the preparation of compounds of Formula I wherein the P3 group is capped with an amide or a sulfonamide, or thiourea, or a sulfamide. The construction of said compounds of Formula I can be achieved using standard conditions for the formation of said P4 functionalities from amines.

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Scheme VI

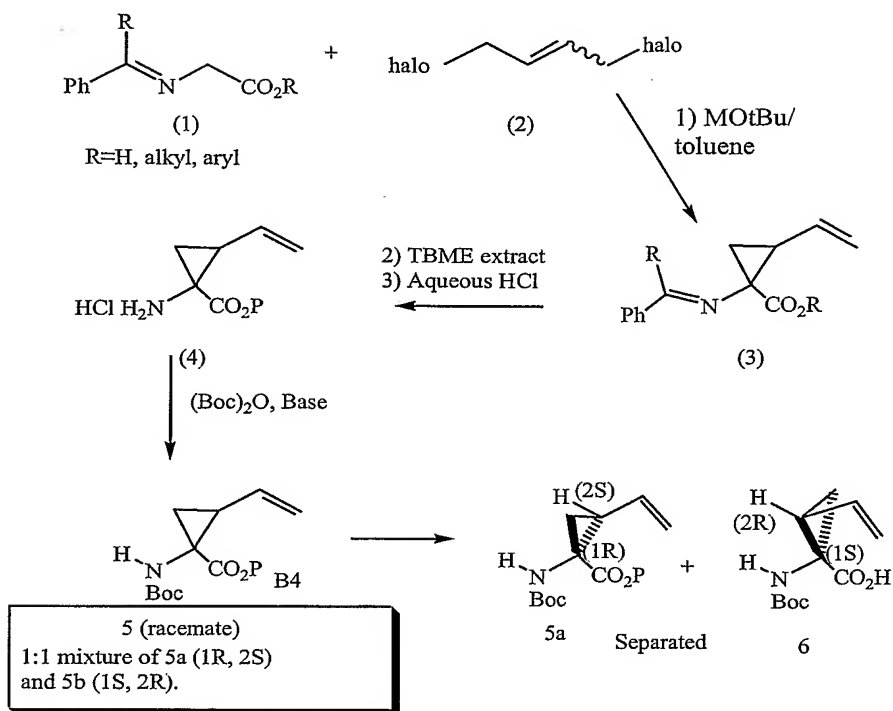
In the construction of compounds of Formula I, the P1' terminus is incorporated into the molecules using one of the general processes outlined above

and described in more detail below. These P1' sulfamides can be prepared in a two step process using chlorosulfonylisocyanate as starting material. Said isocyanate can be hydrolyzed to the corresponding chlorosulfamoyl chloride by treatment with water in a solvent such as THF. The intermediate sulfamoylchloride upon treatment with an amine, in the presence of a base, provides the desired sulfamide derivatives.

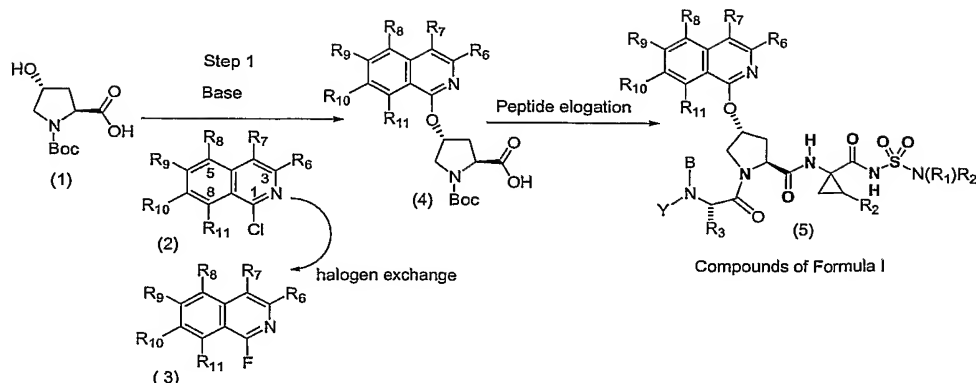


The P1 elements utilized in generating compounds of Formula I are in some cases commercially available, but are otherwise synthesized using the methods described herein and subsequently incorporated into compounds of Formula I using the methods described herein. The substituted P1 cyclopropylamino acids can be synthesized following the general process outline in Scheme VIII.

Treatment of commercially available or easily synthesized imine (1) with 1,4-dihalobutene (2) in presence of a base produces, provides the resulting imine (3). Acid hydrolysis of 3 then provides 4, which has an allyl substituent syn to the carboxyl group as a major product. The amine moiety of 4 can be protected using a Boc group to provide the fully protected amino acid 5. This intermediate is a racemate which can be resolved by an enzymatic process wherein the ester moiety of 5 is cleaved by a protease to provide the corresponding carboxylic acid. Without being bound to any particular theory, it is believed that this reaction is selective in that one of the enantiomers undergoes the reaction at a much greater rate than its mirror image providing for a kinetic resolution of the intermediate racemate. In the examples cited herein, the more preferred stereoisomer for integration into compounds of Formula I is 5a which houses the (1R, 2S) stereochemistry. In the presence of the enzyme, this enantiomer does not undergo ester cleavage and thereby this enantiomer 5a is recovered from the reaction mixture. However, the less preferred enantiomer, 5b with houses the (1S, 2R) stereochemistry undergoes ester cleavage, i.e., hydrolysis, to provide the free acid 6. Upon completion of this reaction, the ester 5a can be separated from the acid product 6 by routine methods such as, for example, aqueous extraction methods or chromatography.

Scheme VIII

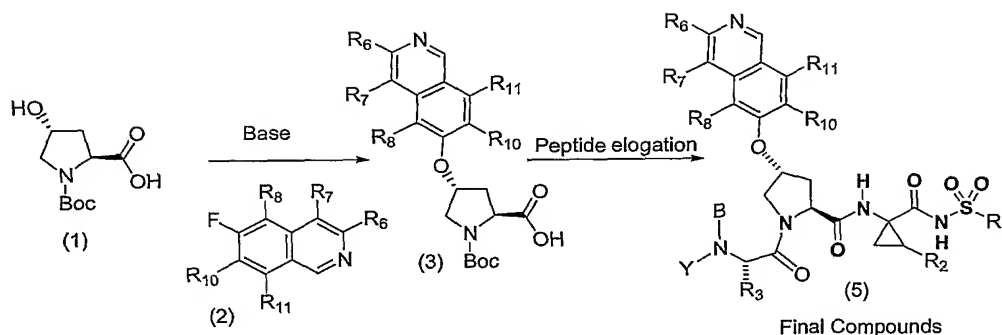
Procedures for making P2 intermediates and compounds of Formula I are shown in the Schemes below. It should be noted that in many cases reactions are depicted for only one position of an intermediate. However, it is to be understood that such reactions could be used to impart modifications to other positions within this intermediate. Moreover, said intermediates, reaction conditions and methods given in the specific examples are broadly applicable to compounds with other substitution patterns. The general Schemes outlined below are followed with examples herein. Both general and specific examples are non-limiting, as for example the isoquinoline nucleus is shown as part of the general scheme, Scheme IX, however, this pathway represents a viable process for the construction of alternate heterocycle substituents as replacements for the isoquinoline element, such as quinolines, or pyridines.

Scheme IX

Scheme IX shows the coupling of an N-protected C4-hydroxyproline moiety with a heterocycle to form intermediate (4) and the subsequent modification of said intermediate (4) to a compound of Formula I by the process of peptide elongation as described herein. It should be noted that in the first step, that is the coupling of the C4-hydroxy proline group with the heteroaryl element, a base is employed. One skilled in the art would recognized that this coupling can be done using bases such as potassium tert-butoxide, or sodium hydride, in solvent such as DMF or DMSO or THF. This coupling to the isoquinoline ring system occurs at the C1 position (numbering for isoquinoline ring system shown in intermediate 2 of Scheme IX) and is directed by the chloro group which is displaced in this process. It should be noted that the alternative leaving groups can be utilized at this position such as a fluoro as shown in the Scheme. Said fluoro intermediates (3) are available from the corresponding chloro compound using literature procedures described herein. It should also be noted that the position of the leaving group (chloro or fluoro) in a given ring system can vary as shown in Scheme X, wherein the leaving group (fluoro in this example) is in the C6 position of the isoquinoline ring system of intermediate (2).

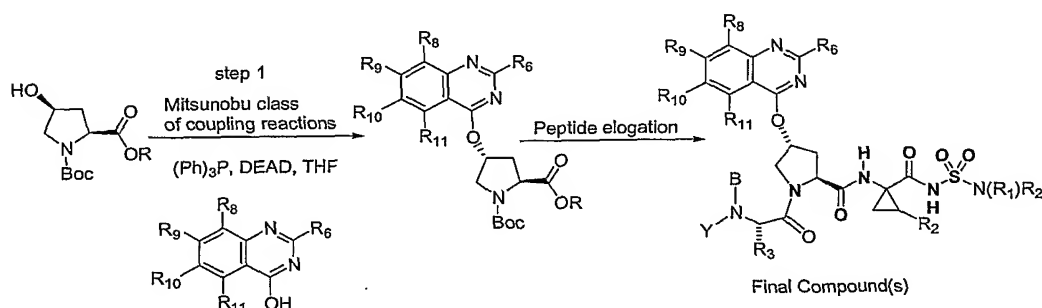
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Scheme X

It should be further noted that the position of the ring heteroatom(s) in intermediates like (2) of Scheme IX and Scheme X is also variable, as defined by the term heterocycle described herein. In Scheme X intermediate (2) can be coupled to a C4 hydroxy proline derivative to provide the P2 element (3). This C6-substituted isoquinoline derivative can be converted to compounds of Formula I using the methods described herein.

10 An alternative to the method described above for the coupling of the C4-hydroxyproline to aromatics and heteroaromatics, is provided in the Mitsunobu reaction as depicted in

Scheme XI

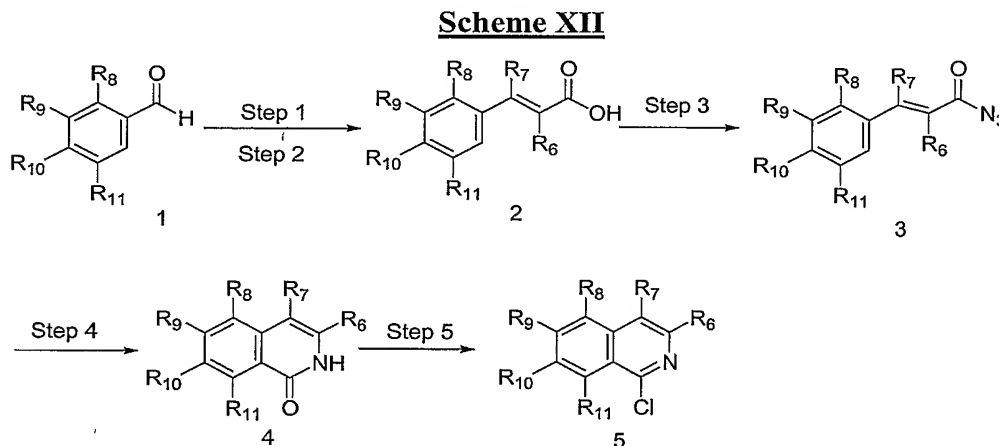
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step 1 of Scheme XI. In this general reaction Scheme a C4-hydroxy proline derivative is coupled to a quinazoline ring system. This reaction makes use of reagents such as triphenylphosphine and DEAD (diethylazodicarboxylate) in aprotic solvents such as THF or dioxane and can be used for the formation of aryl and heteroaryl ethers. Note that in the course of this coupling reaction the

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stereochemistry of the C4 chiral center in the C4-hydroxyproline derivative is inverted and thereby it is necessary to use the C4-hydroxyproline derivative housing the (S) stereochemistry at the C4 position as starting material. (as shown in Scheme XI). It should be noted that numerous modifications and improvements of the Mitsunobu reaction have been described in the literature, the teachings of which are incorporated herein.

In a subset of examples herein, isoquinolines are incorporated into the final compounds and specifically into the P2 region of said compounds. One skilled in the art would recognize that a number of general methods are available for the synthesis of isoquinolines. Moreover, said isoquinolines generated by these methods can be readily incorporated into final compounds of Formula I using the processes described herein. One general methodology for the synthesis of isoquinolines is shown in Scheme XII, wherein cinnamic acid derivatives, shown in general form as structure (2) are

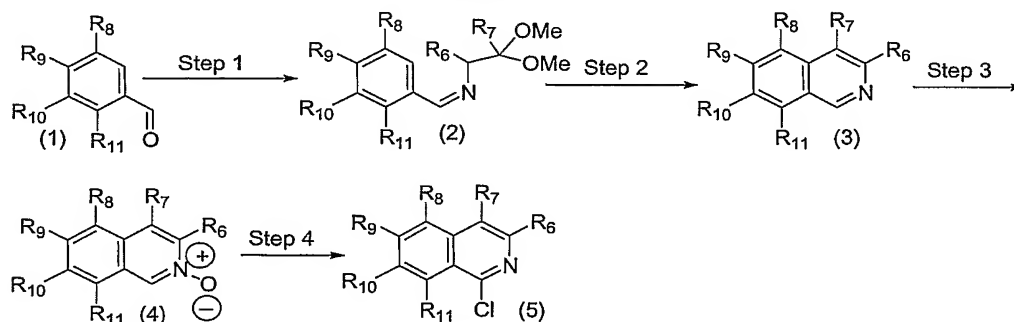


Reference: N. Briet et al, Tetrahedron, 2002, 5761

converted to 1-chloroisoquinolines in a four step process. Said chloroisoquinolines can be subsequently used in coupling reactions to C4-hydroxyproline derivatives as described herein. The conversion of cinnamic acids to chloroquinolines begins with the treatment of cinnamic acid with an alkylchloroformate in the presence of a base. The resulting anhydride is then treated with sodium azide which results in the formation of an acylazide (3) as shown in the Scheme. Alternate methods are available for the formation of acylazides from carboxylic acids as for example said

carboxylic acid can be treated with diphenylphosphorylazide (DPPA) in an aprotic solvent such as methylene chloride in the presence of a base. In the next step of the reaction sequence the acylazide (3) is converted to the corresponding isoquinolone (4) as shown in the Scheme. In the event the acylazide is heated to a temperature of approximately 190 degrees celcius in a high boiling solvent such a diphenylmethane. This reaction is general and provides moderate to good yields of substituted isoquinolone from the corresponding cinnamic acid derivatives. It should noted that said cinnamic acid derivatives are available commercially or can be obtained from the corresponding benzaldehyde (1) derivative by direct condensation with malonic acid or derivatives thereof and also by employing a Wittig reaction. The intermediate isoquinolones (4) of Scheme XII can be converted to the corresponding 1-chloroisoquinoline by treatment with phosphorous oxychloride. This reaction is general and can be applied to any of the isoquinolones, quinolones or additional heterocycles as shown herein to covert a hydroxy substituent to the corresponding chloro compound when said hydroxy is in conjugation with a nitrogen atom in said heterocyclic ring systems.

An alternative method for the synthesis of the isoquinoline ring system is the Pomeranz-Fritsh procedure. This general method is outlined in Scheme XIII. The process begins with the conversion of a benzaldehyde derivative (1) to a functionalized imine (2). Said imine is then converted to the isoquinoline ring system by treatment with acid at elevated

Scheme XIII

Pomeranz-Fritsch synthesis

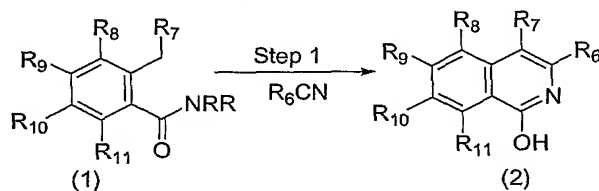
K. Hirao, R. Tsuchiya, Y. Yano, H. Tsue, Heterocycles 42(1) 1996, 415-422

temperature. This isoquinoline synthesis of Scheme XIII is general, and it should be noted that this process is particularly useful in procuring isoquinoline intermediates that are substituted at the C8 position (note: in intermediate (3) of Scheme XIII the C8 position of the isoquinoline ring is substituted with substituent R_{11}). The

- 5 intermediate isoquinolines (3) can be converted to the corresponding 1-chloroquinolines (5) in a two step process as shown. The first step in this sequence is the formation of the isoquinoline N-oxide(4) by treatment of isoquinoline (3) with meta-chloroperbenzoic acid in an aprotic solvent such as dichloromethane. Intermediate (4) can be converted to the corresponding 1-chloroquinoline by
- 10 treatment with phosphorous oxychloride in refluxing chloroform. Note this two step process is general and can be employed to procure chloroisoquinolines and chloroquinolines from the corresponding isoquinolines and quinolines respectively.

- Another method for the synthesis of the isoquinoline ring system is shown in
- 15 Scheme XIV. In this process an ortho-alkylbenzamide derivative (1) is treated with a strong

Scheme XIV

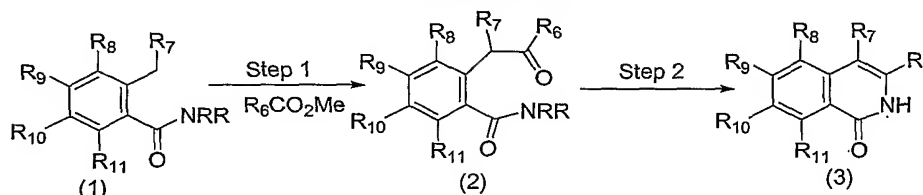


- 20 base such as tert-butyl lithium in a solvent such as THF at low temperature. To this reaction mixture is then added a nitrile derivative, which undergoes an addition reaction with the anion derived from deprotonation of (1), resulting in the formation of (2). This reaction is general and can be used for the formation of substituted isoquinolines. Intermediate (2) of Scheme XIV can be converted to the corresponding
- 25 1-chloroquinoline by the methods described herein.

- An additional method for the synthesis of isoquinolines is shown in Scheme XV. The deprotonation of intermediate (1) using tert-butyl lithium is described above. In the present method however, said intermediate anion is trapped by an ester, resulting in the formation of intermediate (2) as shown below. In a subsequent reaction ketone
- 30 (2) is condensed with ammonium acetate at elevated temperature providing for the

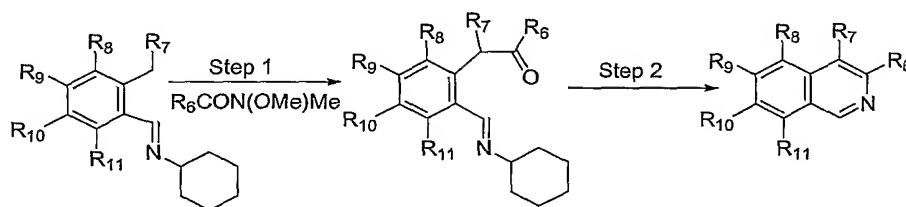
formation of quinolone (3). This reaction is general and can be applied for the construction of substituted isoquinolones which can then be converted to the corresponding 1-chloroisoquinolines as described herein.

5

Scheme XV

Yet an additional method for the construction of isoquinolines is found in Scheme XVI. In the first step of this process an ortho-alkylarylimine derivatives such as (1) is subjected to deprotonation conditions (sec-butyl lithium, THF) and the resulting anion is quenched by

10

Scheme XVI

L. Flippin, J. Muchowski, JOC, 1993, 2631-2632

the addition of an activated carboxylic acid derivative such as a Weinreb amide. The resulting keto imine (2) can be converted to the corresponding isoquinoline by condensation with ammonium acetate at elevated temperatures. This method is general and can be used for the synthesis of substituted isoquinolines. Said isoquinolines can be converted to the corresponding 1-chloroquinoline by the methods described herein.

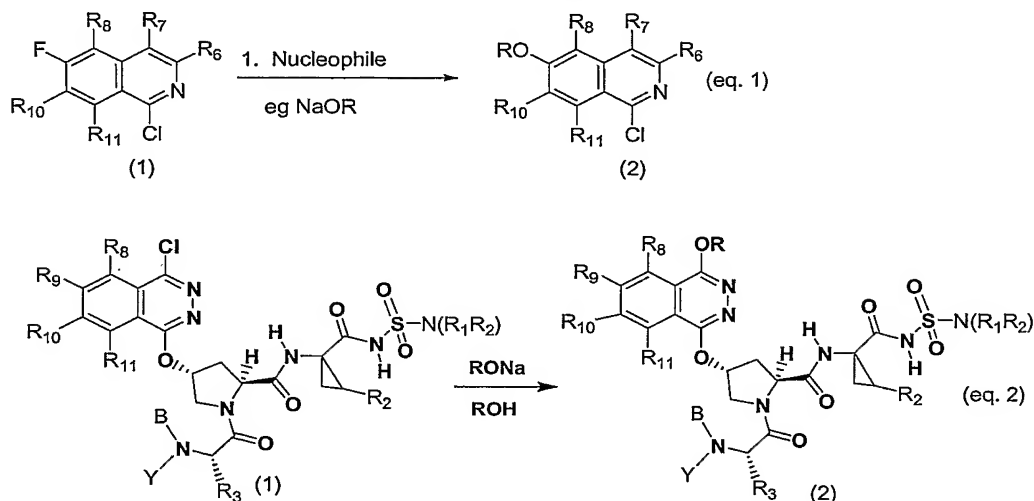
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The heterocycles described herein, and which are incorporated into the compounds of Formula I can be further functionalized. One skilled in the art will recognize that additional functionalization of said heterocycles can be done either before or after incorporation of these functionalities into compounds of Formula I.

25

The following Schemes illustrate this point. For example Scheme XVII shows the conversion of a 1-chloro-

Scheme XVII



6-fluoro-isoquinoline to the corresponding 1-chloro-6-alkoxy-isoquinoline species, by treatment of (1) of (eq.1) with a sodium or potassium alkoxide species in the alcohol solvent from which the alkoxide is derived at room temperature. In some cases it may be necessary to heat the reaction to drive it to completion. Said chloroquinoline can be incorporated into a compound of Formula I using the art described herein.

Modifications of a P2 heterocyclic element can also be done after it's incorporation into compounds of Formula I as shown in (eq.2) of Scheme VXII.

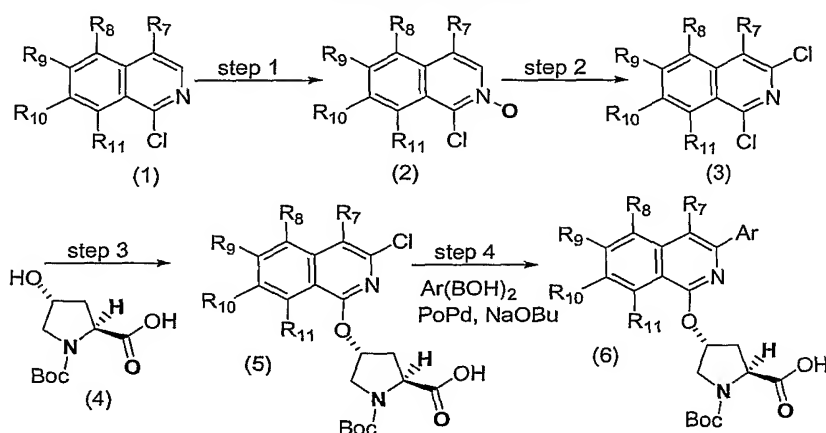
Specifically compounds such as (1) in (eq. 2) which contain a leaving group in the thiazine nucleus can be displaced by a nucleophile such as an alkoxide in solvents such as the corresponding alcohol from which the alkoxide is derived. These reaction can be conducted at room temperature but in some cases it may be necessary to heat the reaction to drive it to completion.

Scheme XVIII provides a general example for the modification of heterocycles as defined herein by employing palladium mediated coupling reactions. Said couplings can be employed to functionalize a heterocycle at each position of the ring system providing said ring is suitably activated or functionalized, as for example

with a chloride as shown in the Scheme. This sequence begins with 1-chloroisoquinoline (1) which upon treatment with metachloroperbenzoic acid can be converted to the corresponding N-oxide (2). Said intermediate (2) can be converted to the corresponding 1,3-dichloroisoquinoline (3) by treatment with phosphorous oxychloride in refluxing chloroform. Intermediate (3) can be coupled with N-Boc-4-hydroxyproline by the methods described herein to provide intermediate (5) as shown in the Scheme. Intermediate (5) can undergo a Suzuki coupling with an aryl boronic acid, in the presence of a palladium reagent and base, and in a solvent such as THF or toluene or DMF to provide the C3-arylisoquinoline intermediate (6).

Heteroarylboronic acids can also be employed in this Pd mediated coupling process to provide C3-heteroarylisoquinolines. Intermediate (6) can be converted into final compounds of Formula I by the methods described herein.

Scheme XVIII



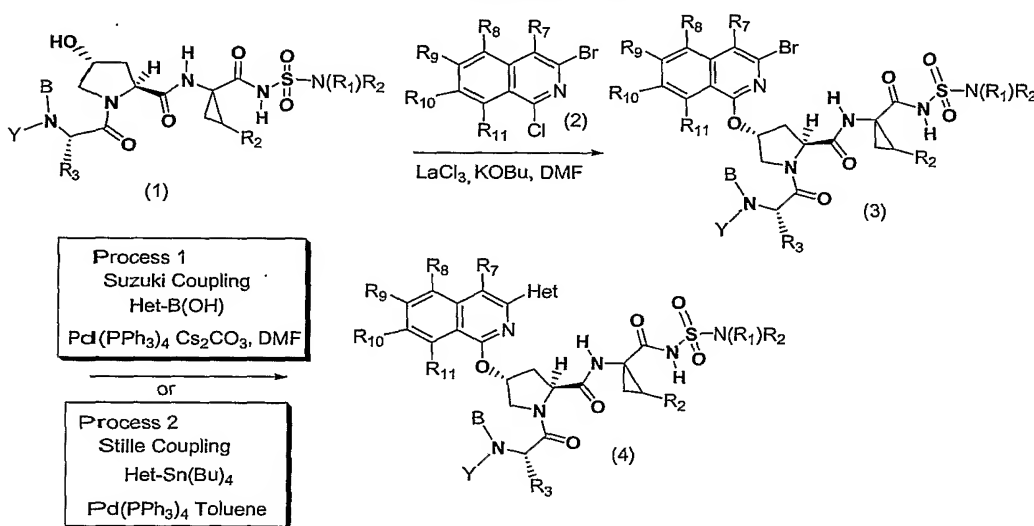
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Palladium mediated couplings of heteroaryl systems with aryl or heteroaryl elements can also be employed at a later synthetic stage in the construction of compounds of Formula I as shown in Scheme IXX. Therein tripeptide acylsulfonamide intermediate (1) is coupled to a 1-chloro-3-bromoisoquinoline (2) using the previously described process of alkoxide displacement of an heteroarylhalo moiety to provide intermediate (3). The coupling of (1) and (2) is most efficient in the presence of a catalyst such as lanthanum chloride as described herein. The isoquinoline ring system of intermediate (3) can be further functionalized by employing either Suzuki couplings (Process 1: subjecting (3) to heteroaryl or aryl

25

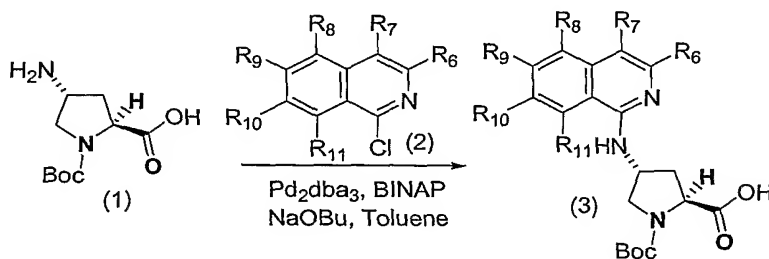
boronic acids in the presence of a palladium catalyst such as palladium tetra(triphenylphosphine) and a base such as cesium carbonate in solvents such as DMF) or Stille couplings (Process 2: subjecting (3) to heteraryl or aryl tin derivatives in the presence of palladium catalyst such as palladium tetra(triphenylphosphine) in solvents such as toluene).

Scheme IXX

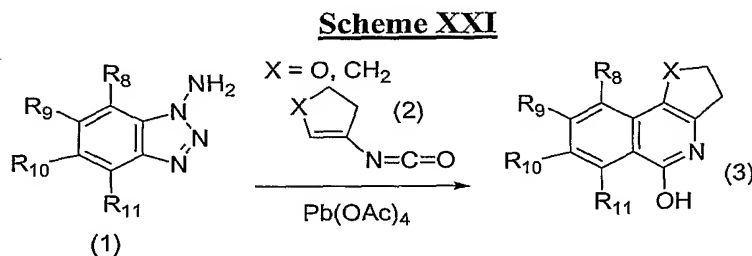


Palladium reactions can also be employed to couple C4-amino proline elements with functionalized heterocycles. Scheme XX shows intermediate (1) coupling with a functionalized isoquinoline in the presence of a palladium catalyst and a base in a solvent such as toluene. Intermediates like (3) can be converted to compounds of Formula I using the methods described herein.

Scheme XX



The construction of functionalized isoquinoline ring systems is also possible employing [4+2] cycloaddition reactions. For example (Scheme XXI) the use of vinyl isocyanates (1) in cycloaddition reactions with benzyne precursors (2) provides functionalized isoquinolones (3). Said isoquinolones can be incorporated into
5 compounds of Formula I using the methods described herein.



10 Compounds of the invention can also be prepared by utilizing methods known to those skilled in the art, such as, for example, the methods described in patent application WO 03/099274, published December 3, 2003. For example, WO 03/099274 provides extensive examples and teachings on the preparation of proline derivatives as intermediates and these intermediates can be used in the preparation of
15 compound of Formula 1.

The present invention also provides compositions comprising a compound of the present invention, or a pharmaceutically acceptable salt, solvate or prodrug thereof, and a pharmaceutically acceptable carrier. Pharmaceutical compositions of
20 the present invention comprise a therapeutically effective amount of a compound of the invention, or a pharmaceutically acceptable salt, solvate or prodrug thereof, and a pharmaceutically acceptable carrier, with a pharmaceutically acceptable carrier, e.g., excipient, or vehicle diluent.

25 The active ingredient, i.e., compound, in such compositions typically comprises from 0.1 weight percent to 99.9 percent by weight of the composition, and often comprises from about 5 to 95 weight percent.

Thus, in one aspect of the invention, there is provided a composition comprising the compound of formula 1 and a pharmaceutically acceptable carrier. Preferably, the composition further comprises a compound having anti-HCV activity. As used herein, the term “anti-HCV activity” means the compound is effective to inhibit the function of a target selected from the group consisting of HCV metalloprotease, HCV serine protease, HCV polymerase, HCV helicase, HCV NS4B protein, HCV entry, HCV assembly, HCV egress, HCV NS5A protein, IMPDH and a nucleoside analog for the treatment of an HCV infection. Often, the other compound having anti-HCV activity is effective to inhibit the function of target in the HCV life cycle other than the HCV serine protease.

In one preferred aspect, the compound having anti-HCV activity is an interferon. Preferably, the interferon is selected from the group consisting of interferon alpha 2B, pegylated interferon alpha, consensus interferon, interferon alpha 2A and lymphoblastoid interferon tau.

In another aspect of the invention, the compound having anti-HCV activity is selected from the group consisting of interleukin 2, interleukin 6, interleukin 12, a compound that enhances the development of a type 1 helper T cell response, interfering RNA, anti-sense RNA, Imiquimod, ribavirin, an inosine 5'-monophosphate dehydrogenase inhibitor, amantadine, and rimantadine.

In one preferred aspect of the invention, the composition comprises a compound of the invention, an interferon and ribavirin.

In another preferred aspect of the invention, the compound having anti-HCV activity is a small molecule compound. As used herein, the term “small molecule compound” means a compound having a molecular weight of less than 1,500 daltons, preferably less than 1000 daltons. Preferably, the small molecule compound is effective to inhibit the function of a target selected from the group consisting of HCV metalloprotease, HCV serine protease, HCV polymerase, HCV helicase, HCV NS4B protein, HCV entry, HCV assembly, HCV egress, HCV NS5A protein, inosine

monophosphate dehydrogenase ("IMPDH") and a nucleoside analog for the treatment of an HCV infection.

Certain illustrative HCV inhibitor compounds which can be administered with the compounds of the present invention include those disclosed in the following publications: WO 02/04425 A2 published January 17, 2002, WO 03/007945 A1 published January 30, 2003, WO 03/010141 A2 published February 6, 2003, WO 03/010142 A2 published February 6, 2003, WO 03/010143 A1 published February 6, 2003, WO 03/000254 A1 published January 3, 2003, WO 01/32153 A2 published May 10, 2001, WO 00/06529 published February 10, 2000, WO 00/18231 published April 6, 2000, WO 00/10573 published March 2, 2000, WO 00/13708 published March 16, 2000, WO 01/85172 A1 published November 15, 2001, WO 03/037893 A1 published May 8, 2003, WO 03/037894 A1 published May 8, 2003, WO 03/037895 A1 published May 8, 2003, WO 02/100851 A2 published December 19, 2002, WO 02/100846 A1 published December 19, 2002, EP 1256628 A2 published November 13, 2002, WO 99/01582 published January 14, 1999, WO 00/09543 published February 24, 2000.

Table 1 below lists some illustrative examples of compounds that can be administered with the compounds of this invention. The compounds of the invention can be administered with other anti-HCV activity compounds in combination therapy, either jointly or separately, or by combining the compounds into a composition.

TABLE 1

| Brand Name | Type of Inhibitor or Target | Source Company |
|-------------------|------------------------------------|---|
| Omega IFN | IFN- ω | BioMedicines Inc., Emeryville, CA |
| BILN-2061 | serine protease inhibitor | Boehringer Ingelheim Pharma KG, Ingelheim, Germany |
| Summetrel | antiviral | Endo Pharmaceuticals Holdings Inc., Chadds Ford, PA |

| Brand Name | Type of Inhibitor or Target | Source Company |
|-----------------------|---|--|
| Roferon A | IFN- α 2a | F. Hoffmann-La Roche LTD, Basel, Switzerland |
| Pegasys | PEGylated IFN- α 2a | F. Hoffmann-La Roche LTD, Basel, Switzerland |
| Pegasys and Ribavirin | PEGylated IFN- α 2a/ribavirin | F. Hoffmann-La Roche LTD, Basel, Switzerland |
| CellCept | HCV IgG immunosuppressant | F. Hoffmann-La Roche LTD, Basel, Switzerland |
| Wellferon | lymphoblastoid IFN- α n1 | GlaxoSmithKline plc, Uxbridge, UK |
| Albuferon - α | albumin IFN- α 2b | Human Genome Sciences Inc., Rockville, MD |
| Levovirin | ribavirin | ICN Pharmaceuticals, Costa Mesa, CA |
| IDN-6556 | caspase inhibitor | Idun Pharmaceuticals Inc., San Diego, CA |
| IP-501 | antifibrotic | Indevus Pharmaceuticals Inc., Lexington, MA |
| Actimmune | INF- γ | InterMune Inc., Brisbane, CA |
| Infergen A | IFN alfacon-1 | InterMune Pharmaceuticals Inc., Brisbane, CA |
| ISIS 14803 | antisense | ISIS Pharmaceuticals Inc, Carlsbad, CA/Elan Pharmaceuticals Inc., New York, NY |
| JTK-003 | RdRp inhibitor | Japan Tobacco Inc., Tokyo, Japan |
| Pegasys and Ceplene | PEGylated IFN- α 2a/immune modulator | Maxim Pharmaceuticals Inc., San Diego, CA |
| Ceplene | immune modulator | Maxim Pharmaceuticals Inc., San Diego, CA |
| Civacir | HCV IgG immunosuppressant | Nabi Biopharmaceuticals Inc., Boca Raton, FL |
| Intron A and Zadaxin | IFN- α 2b/ α 1-thymosin | RegeneRx Biopharmaceuticals Inc., Bethesda, MD/SciClone Pharmaceuticals Inc, San Mateo, CA |
| Levovirin | IMPDH inhibitor | Ribapharm Inc., Costa Mesa, CA |
| Viramidine | IMPDH inhibitor | Ribapharm Inc., Costa |

| Brand Name | Type of Inhibitor or Target | Source Company |
|-------------------------|--------------------------------------|---|
| | | Mesa, CA |
| Heptazyme | ribozyme | Ribozyme Pharmaceuticals Inc., Boulder, CO |
| Intron A | IFN- α 2b | Schering-Plough Corporation, Kenilworth, NJ |
| PEG-Intron | PEGylated IFN- α 2b | Schering-Plough Corporation, Kenilworth, NJ |
| Rebetron | IFN- α 2b/ribavirin | Schering-Plough Corporation, Kenilworth, NJ |
| Ribavirin | ribavirin | Schering-Plough Corporation, Kenilworth, NJ |
| PEG-Intron / Ribavirin | PEGylated IFN- α 2b/ribavirin | Schering-Plough Corporation, Kenilworth, NJ |
| Zadazim | immune modulator | SciClone Pharmaceuticals Inc., San Mateo, CA |
| Rebif | IFN- β 1a | Serono, Geneva, Switzerland |
| IFN- β and EMZ701 | IFN- β and EMZ701 | Transition Therapeutics Inc., Ontario, Canada |
| T67 | β -tubulin inhibitor | Tularik Inc., South San Francisco, CA |
| VX-497 | IMPDH inhibitor | Vertex Pharmaceuticals Inc., Cambridge, MA |
| VX-950/LY-570310 | serine protease inhibitor | Vertex Pharmaceuticals Inc., Cambridge, MA/ Eli Lilly and Co. Inc., Indianapolis, IN |
| Omniferon | natural IFN- α | Viragen Inc., Plantation, FL |
| XTL-002 | monoclonal antibody | XTL Biopharmaceuticals Ltd., Rehovot, Isreal |

The pharmaceutical compositions of this invention may be administered orally, parenterally or via an implanted reservoir. Oral administration or

5 administration by injection are preferred. In some cases, the pH of the formulation

may be adjusted with pharmaceutically acceptable acids, bases or buffers to enhance the stability of the formulated compound or its delivery form. The term parenteral as used herein includes subcutaneous, intracutaneous, intravenous, intramuscular, intra-articular, intrasynovial, intrasternal, intrathecal, and intralesional injection or infusion techniques.

When orally administered, the pharmaceutical compositions of this invention may be administered in any orally acceptable dosage form including, but not limited to, capsules, tablets, and aqueous suspensions and solutions. In the case of tablets for oral use, carriers which are commonly used include lactose and corn starch. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions are administered orally, the active ingredient is combined with emulsifying and suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added. Other suitable carriers for the above noted compositions can be found in standard pharmaceutical texts, e.g. in "Remington's Pharmaceutical Sciences", 19th ed., Mack Publishing Company, Easton, Penn., 1995.

The pharmaceutical compositions can be prepared by known procedures using well-known and readily available ingredients. The compositions of this invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient by employing procedures well known in the art. In making the compositions of the present invention, the active ingredient will usually be admixed with a carrier, or diluted by a carrier, or enclosed within a carrier which may be in the form of a capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semi-solid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, beadlets, lozenges, sachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions, sterile packaged powders and the like. Further details concerning the design and

preparation of suitable delivery forms of the pharmaceutical compositions of the invention are known to those skilled in the art.

Dosage levels of between about 0.01 and about 1000 milligram per kilogram ("mg/kg") body weight per day, preferably between about 0.5 and about 250 mg/kg body weight per day of the compounds of the invention are typical in a monotherapy for the prevention and treatment of HCV mediated disease. Typically, the pharmaceutical compositions of this invention will be administered from about 1 to about 5 times per day or alternatively, as a continuous infusion. Such administration can be used as a chronic or acute therapy. The amount of active ingredient that may be combined with the carrier materials to produce a single dosage form will vary depending upon the host treated and the particular mode of administration.

As the skilled artisan will appreciate, lower or higher doses than those recited above may be required. Specific dosage and treatment regimens for any particular patient will depend upon a variety of factors, including the activity of the specific compound employed, the age, body weight, general health status, sex, diet, time of administration, rate of excretion, drug combination, the severity and course of the infection, the patient's disposition to the infection and the judgment of the treating physician. Generally, treatment is initiated with small dosages substantially less than the optimum dose of the peptide. Thereafter, the dosage is increased by small increments until the optimum effect under the circumstances is reached. In general, the compound is most desirably administered at a concentration level that will generally afford antivirally effective results without causing any harmful or deleterious side effects.

When the compositions of this invention comprise a combination of a compound of the invention and one or more additional therapeutic or prophylactic agent, both the compound and the additional agent are usually present at dosage levels of between about 10 to 100%, and more preferably between about 10 and 80% of the dosage normally administered in a monotherapy regimen.

When these compounds or their pharmaceutically acceptable enantiomers, diastereomers, salts, solvates or prodrugs are formulated together with a pharmaceutically acceptable carrier, the resulting composition may be administered in vivo to mammals, such as man, to inhibit HCV serine protease or to treat or
5 prevent HCV virus infection.

Accordingly, another aspect of this invention provides methods of inhibiting HVC serine protease activity in patients by administering a compound of the present invention or a pharmaceutically acceptable salt or solvate thereof.
10

In one aspect of the invention, there is provided a method of inhibiting the function of the HCV serine protease comprising contacting the HCV serine protease with a compound of the invention. In another aspect, there is provided a method of treating an HCV infection in a patient, comprising administering to the patient a
15 therapeutically effective amount of the compound of the invention, or a pharmaceutically acceptable solvate, prodrug or salt thereof.

Preferably, the method of administering the compound is effective to inhibit the function of the HCV serine protease. In a preferred aspect, the method further
20 comprises administering another compound having anti-HCV activity (as described above) prior to, after or concurrently with a compound of the invention.

The compounds of the invention may also be used as laboratory reagents. Compounds may be instrumental in providing research tools for designing of viral
25 replication assays, validation of animal assay systems and structural biology studies to further enhance knowledge of the HCV disease mechanisms. Further, the compounds of the present invention are useful in establishing or determining the binding site of other antiviral compounds, for example, by competitive inhibition.

30 The compounds of this invention may also be used to treat or prevent viral contamination of materials and therefore reduce the risk of viral infection of laboratory or medical personnel or patients who come in contact with such materials,

e.g., blood, tissue, surgical instruments and garments, laboratory instruments and garments, and blood collection or transfusion apparatuses and materials.

Further, the compounds and compositions of the invention can be used for the manufacture of a medicament for treating an HCV infection in a patient.

EXAMPLES

The specific examples that follow illustrate the syntheses of the compounds of the instant invention, and are not to be construed as limiting the invention in sphere or scope. The methods may be adapted to variations in order to produce compounds embraced by this invention but not specifically disclosed. Further, variations of the methods to produce the same compounds in somewhat different manner will also be evident to one skilled in the art.

Solution percentages express a weight to volume relationship, and solution ratios express a volume to volume relationship, unless stated otherwise. Nuclear magnetic resonance (NMR) spectra were recorded either on a Bruker 300, 400 or 500 MHz spectrometer; the chemical shifts (δ) are reported in parts per million. Flash chromatography was carried out on silica gel (SiO_2) according to Still's flash chromatography technique (W.C. Still et al., J. Org. Chem., (1978), 43, 2923).

All Liquid Chromatography (LC) data were recorded on a Shimadzu LC-10AS liquid chromatograph using a SPD-10AV UV-Vis detector and Mass Spectrometry (MS) data were determined with a Micromass Platform for LC in electrospray mode (ES+).

Unless otherwise noted, in the following examples each compound was analyzed by LC/MS, using one of seven methodologies, having the following conditions.

Columns: (Method A) - YMC ODS S7 C18 3.0x50 mm
(Method B) - YMC ODS-A S7 C18 3.0x50 mm
(Method C) - YMC S7 C18 3.0x50 mm

(Method D) - YMC Xterra ODS S7 3.0x50 mm

(Method E) - YMC Xterra ODS S7 3.0x50 mm

(Method F) - YMC ODS-A S7 C18 3.0x50 mm

(Method G) - YMC C18 S5 4.6x50 mm]

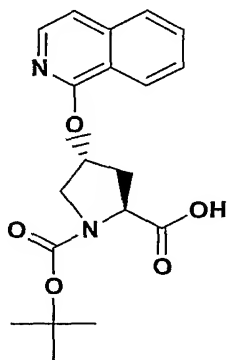
- 5 Gradient: 100% Solvent A/0% Solvent B to
0% Solvent A/100% Solvent B
Gradient time: 2 min. (A, B, D, F, G); 8 min. (C, E)
Hold time: 1 min. (A, B, D, F, G); 2 min. (C, E)
Flow rate: 5 milliliters/min ("mL/min")
- 10 Detector Wavelength: 220 nanometer ("nm")
Solvent A: 10% MeOH / 90% H₂O / 0.1% TFA
Solvent B: 10% H₂O / 90% MeOH / 0.1% TFA.

- The abbreviations used in the present application, including particularly in the
- 15 illustrative examples which follow, are well-known to those skilled in the art. Some of the abbreviations used are as follows:

| | |
|-------------------|---|
| rt | room temperature |
| Boc | tert-butyloxycarbonyl |
| 20 DMSO | dimethylsulfoxide |
| EtOAc | ethyl acetate |
| t-BuOK | potassium t-butoxide |
| Et ₂ O | diethyl ether |
| TBME | tert-butylmethyl ether |
| 25 THF | tetrahydrofuran |
| CDI | carbonyldiimidazole |
| DBU | 1,8-diazabicyclo[5.4.0]undec-7-ene |
| TFA | trifluoroacetic acid |
| NMM | N-methylmorpholine |
| 30 HATU | O-7-azabenzotriazol-1-yl |
| HBTU | O-{1H-benzotriazol-1-yl)-N,N,N',N'- tetramethyluronium hexafluorophosphate |
| HOBT | N-hydroxybenzotriazole |

| | | |
|----|--------|--|
| | PyBrop | bromo-bis-pyrrolidine-phosphonium hexafluorophosphate |
| | DMF | dimethylformamide |
| | MeOH | methanol |
| 5 | EDTA | ethylenediaminetetraacetic acid |
| | HRMS | high resolution mass spectrometry |
| | DMAP | 4-dimethylaminopyridine |
| | DIPEA | diisopropylethylamine |
| | DCM | dichloromethane |
| 10 | DCE | dichloroethane |

15 Preparation of Intermediates:

Example 1**Preparation of 4-(Isoquinolin-1-yloxy)-pyrrolidine-1,2-dicarboxylic acid
1-tert-butyl ester**

20

A suspension of Boc-L-4-hydroxyproline (*N*-Boc (2*S*,4*R*)-hydroxyproline) (3.85 g, 16.6 millimole ("mmol") (CHEM-IMPEX International) in DMSO (60 mL) was cooled in an ice bath for ~ 3 minutes, and then *t*-BuOK (4.66 g, 41.5 mmol) was added. Stirring and cooling in the ice bath was continued for a few minutes until a solid mass formed. At this time the reaction mixture was allowed to warm to rt over 1.5 h to give a clear colorless solution. 1-Chloroisoquinoline (3.0 g, 18.3 mmol) was

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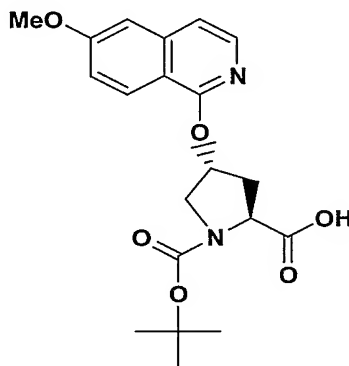
added in two portions 10 min apart. The reaction was stirred for 24 h at rt. The dark reaction mixture was partitioned between ether (200 mL) and water (600 mL). The aqueous phase was acidified to pH 4 using 4N HCl. The resulting milky yellow solution was extracted with ether (4 x 200 mL). The combined ether extracts were
5 dried (MgSO₄), filtered, and concentrated in vacuo to give a golden oil. Flash chromatography (2-4% MeOH/CH₂Cl₂) gave 5.69 g (96%) of 4-(isoquinolin-1-yloxy)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester as an off-white solid: LC-MS (Method A, retention time: 3.06 min), MS *m/z* 359 (M⁺+1).

10

Example 2

Preparation of 4-(6-Methoxy-isoquinolin-1-yloxy)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester

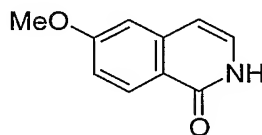
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Step 1 of Example 2

Preparation of 6-Methoxy-2*H*-isoquinolin-1-one

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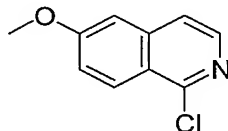


6-Methoxy-2*H*-isoquinolin-1-one

To a solution of 3-methoxy cinnamic acid (11.04 g, 62 mmol), triethylamine (12.52 g, 124 mmol) in acetone (80 mL) was added ethyl chloroformate dropwise at 0°C. After stirring at this temperature for 1 h, aqueous NaN₃ (6.40 g, 100 mmol in 35 mL H₂O) was added dropwise and the reaction mixture was stirred for 16 h at the ambient temperature. Water (100 mL) was added to the mixture and the volatile was removed in vacuo. The resulting slurry was extracted with toluene (3X50 mL) and the combined organic layers were dried over MgSO₄. This dried solution was added dropwise to a heated solution of diphenylmethane (50 mL) and tributylamine (30 mL) at 190°C. The toluene was distilled off as added. After complete addition, the reaction temperature was raised to 210°C for 2 h. After cooling, the precipitated product was collected by filtration, washed with hexane (2X50 mL), dried to yield a white solid (5.53 g, 51%), MS *m/z* 176 (M⁺+H).

Step 2 of Example 2

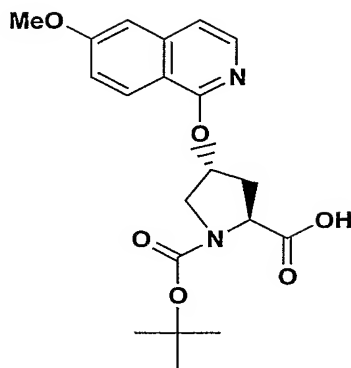
Preparation of 1-chloro-6 methoxy isoquinoline



6-Methoxy-2*H*-isoquinolin-1-one in POCl₃ was heated to gentle reflux for 3 h then evaporated *in vacuo*. The residue was poured into ice-water (20 mL) and neutralized to pH 10 with 10 M NaOH. Extracted with CHCl₃. The organic layer was washed with brine, dried over MgSO₄, filtered, evaporated. The residue was purified by flash chromatography (hexane-EtOAc) to afford a white solid. ¹H NMR (CD₃OD) δ 3.98 (s, 3H), 7.34-7.38 (m, 2 H), 7.69 (d, *J*=5.5 Hz, 1H), 8.10 (d, *J*=6.0 Hz, 1H), 8.23 (d, *J*=9.5 Hz, 1H), MS *m/z* 194 (M⁺+H).

Step 3 of Example 2

Preparation of 4-(6-Methoxy-isoquinolin-1-yloxy)-pyrrolidine-1,2-dicarboxylic acid 1-*tert*-butyl ester



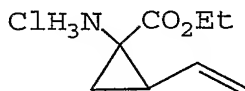
As described in Example 1 except that 1-chloro-6-methoxy-isoquinoline was used in place of 1-chloro-isoquinoline. ^1H NMR (400 MHz, CD_3OD) δ 1.41 (m, 9 H), 2.44 (m, 1 H), 2.75 (m, 1 H), 3.82-3.92 (m, 2 H), 3.96 (s, 3 H), 4.45 (m, 1 H), 5.50 (m, 1 H), 7.20 (m, 1 H), 7.27 (d, $J=5.14$ Hz, 1 H), 7.41 (d, $J=2.45$ Hz, 1 H), 7.48-7.59 (m, 3 H), 8.00-8.05 (m, 2 H), 8.08 (d, $J=9.29$ Hz, 1 H).
LC-MS (retention time: 2.140 min.), MS m/z 465 (MH^+).

Example 3

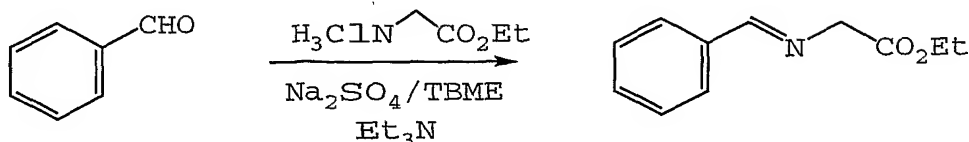
Preparation of P1 elements for incorporation into compounds of Formula

I

I. Preparation of racemic (1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester hydrochloride (Method A and Method B) and chiral resolution of this racemate for the preparation of *N*-(1*R*, 2*S*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester hydrochloride (Method C)



The named compound was made racemic by each of the following methods A and B. This racemate could also be resolved to afford chiral Boc-(1*R*, 2*S*)-1-amino-2-vinylcyclopropyl carboxylic acid ester which was deprotected under acid conditions to afford (1*R*, 2*S*)-1-amino-2-vinylcyclopropane carboxylic acid ester hydrochloride (Method C).

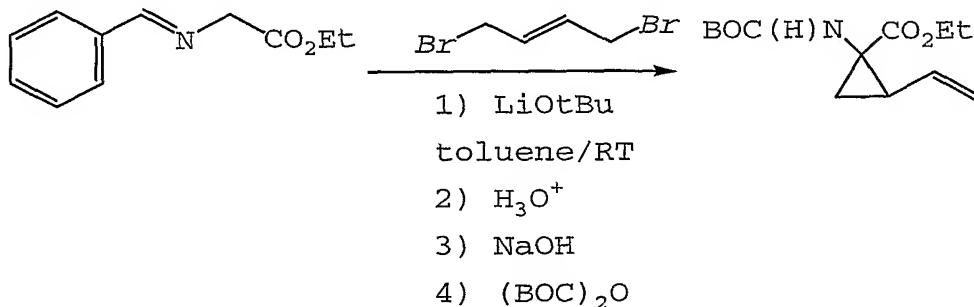
Method A**A.1 Preparation of *N*-Benzyl Imine of Glycine Ethyl Ester**

5

Glycine ethyl ester hydrochloride (303.8 g, 2.16 mole) was suspended in *tert*-butylmethyl ether (1.6 L). Benzaldehyde (231 g, 2.16 mole) and anhydrous sodium sulfate (154.6 g, 1.09 mole) were added and the mixture cooled to 0 °C using an ice-water bath. Triethylamine (455 mL, 3.26 mole) was added dropwise over 30 min and the mixture stirred for 48 h at rt. The reaction was then quenched by addition of ice-cold water (1 L) and the organic layer was separated. The aqueous phase was extracted with *tert*-butylmethyl ether (0.5 L) and the combined organic phases washed with a mixture of saturated aqueous NaHCO₃ (1 L) and brine (1 L). The solution was dried over MgSO₄, concentrated in vacuo to afford 392.4 g of the *N*-benzyl imine product as a thick yellow oil that was used directly in the next step. ¹H NMR (CDCl₃, 300 MHz) δ 1.32 (t, *J*=7.1 Hz, 3H), 4.24 (q, *J*=7.1 Hz, 2H), 4.41 (d, *J*=1.1 Hz, 2H), 7.39-7.47 (m, 3H), 7.78-7.81 (m, 2H), 8.31 (s, 1H).

A.2 Preparation of racemic *N*-Boc-(1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester

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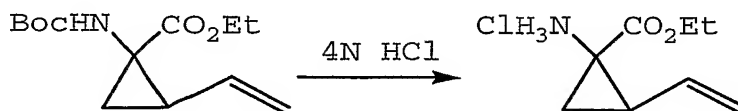


To a suspension of lithium *tert*-butoxide (84.06 g, 1.05 mol) in dry toluene (1.2 L), was added dropwise a mixture of the *N*-benzyl imine of glycine ethyl ester (100.4 g,

25

0.526 mol) and *trans*-1,4-dibromo-2-butene (107.0 g, 0.500 mol) in dry toluene (0.6 L) over 60 min. After completion of the addition, the deep red mixture was quenched by addition of water (1 L) and *tert*-butylmethyl ether (TBME, 1 L). The aqueous phase was separated and extracted a second time with TBME (1 L). The organic phases were combined, 1 N HCl (1 L) was added and the mixture stirred at room temperature for 2 h. The organic phase was separated and extracted with water (0.8 L). The aqueous phases were then combined, saturated with salt (700 g), TBME (1 L) was added and the mixture cooled to 0 °C. The stirred mixture was then basified to pH 14 by the dropwise addition of 10 N NaOH, the organic layer separated, and the aqueous phase extracted with TBME (2 x 500 mL). The combined organic extracts were dried (MgSO₄) and concentrated to a volume of 1L. To this solution of free amine, was added BOC₂O or di-*tert*-butyldicarbonate (131.0 g, 0.6 mol) and the mixture stirred 4 days at rt. Additional di-*tert*-butyldicarbonate (50 g, 0.23 mol) was added to the reaction, the mixture refluxed for 3 h, and was then allowed cool to room temperature overnight. The reaction mixture was dried over MgSO₄ and concentrated in vacuo to afford 80 g of crude material. This residue was purified by flash chromatography (2.5 Kg of SiO₂, eluted with 1% to 2% MeOH/CH₂Cl₂) to afford 57 g (53%) of racemic *N*-Boc-(1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester as a yellow oil which solidified while sitting in the refrigerator: ¹H NMR (CDCl₃, 300 MHz) δ 1.26 (t, *J*=7.1 Hz, 3H), 1.46 (s, 9H), 1.43-1.49 (m, 1H), 1.76-1.82 (br m, 1H), 2.14 (q, *J*=8.6 Hz, 1H), 4.18 (q, *J*=7.2 Hz, 2H), 5.12 (dd *J*=10.3, 1.7 Hz, 1H), 5.25 (br s, 1H), 5.29 (dd, *J*=17.6, 1.7 Hz, 1H), 5.77 (ddd, *J*=17.6, 10.3, 8.9 Hz, 1H); MS *m/z* 254.16 (M-1)

A.3 Preparation of Racemic (1*R*,2*S*)/(1*S*,2*R*) 1-amino-2-vinylcyclopropane carboxylic acid ethyl ester hydrochloride



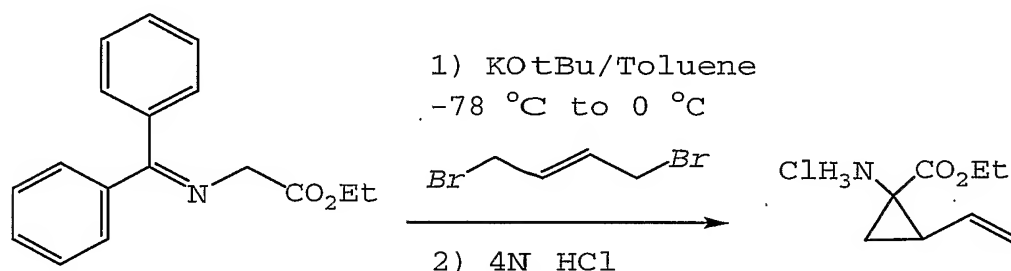
N-Boc-(1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester (9.39 g, 36.8 mmol) was dissolved in 4 N HCl/dioxane (90ml, 360 mmol) and was

stirred for 2 h at rt. The reaction mixture was concentrated to supply (1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester hydrochloride in quantitative yield (7 g, 100%). ¹H NMR (methanol-*d*₄) δ 1.32 (t, *J*=7.1, 3H), 1.72 (dd, *J*=10.2, 6.6 Hz, 1H), 1.81 (dd, *J*=8.3, 6.6 Hz, 1H), 2.38 (q, *J*=8.3 Hz, 1H), 4.26-4.34 (m, 2H),
 5 5.24 (dd, 10.3, 1.3 Hz, 1H) 5.40 (d, *J*=17.2, 1H), 5.69-5.81 (m, 1H).

Method B

Preparation of Racemic (1*R*,2*S*)/(1*S*,2*R*) 1-amino-2-vinylcyclopropane carboxylic acid ethyl ester hydrochloride

10

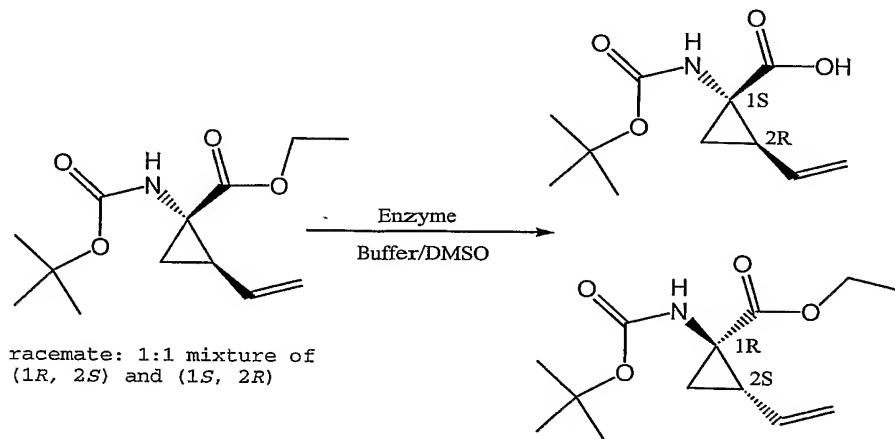


To a solution of potassium *tert*-butoxide (11.55 g, 102.9 mmol) in THF (450 mL) at -78 °C was added the commercially available *N,N*-dibenzyl imine of glycine ethyl ester (25.0 g, 93.53 mmol) in THF (112 mL). The reaction mixture was warmed to 0 °C, stirred for 40 min, and was then cooled back to -78 °C. To this solution was
 15 added *trans*-1,4-dibromo-2-butene (20.0 g, 93.50 mmol), the mixture stirred for 1 h at 0°C and was cooled back to -78°C. Potassium *tert*-butoxide (11.55 g, 102.9 mmol) was added, the mixture immediately warmed to 0°C, and was stirred one more hour
 20 before concentrating in vacuo. The crude product was taken up in Et₂O (530 mL), 1N aq. HCl solution (106 mL, 106 mmol) added and the resulting biphasic mixture stirred for 3.5 h at rt. The layers were separated and the aqueous layer was washed with Et₂O (2x) and basified with a saturated aq. NaHCO₃ solution. The desired amine was extracted with Et₂O (3x) and the combined organic extract was washed
 25 with brine, dried (MgSO₄), and concentrated in vacuo to obtain the free amine. This material was treated with a 4N HCl solution in dioxane (100 mL, 400 mmol) and concentrated to afford (1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester hydrochloride as a brown semisolid (5.3 g, 34% yield) identical to the

material obtained from procedure A, except for the presence of a small unidentified aromatic impurity (8%).

Method C

Resolution of *N*-Boc-(1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester

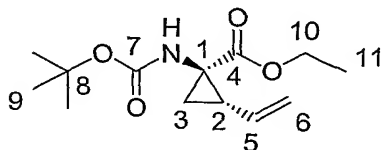


Resolution A

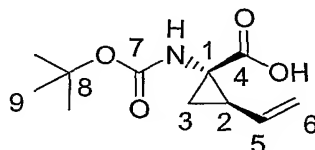
- 10 To an aqueous solution of sodium phosphate buffer (0.1 M, 4.25 liter ("L"), pH 8) housed in a 12 Liter jacked reactor, maintained at 39°C, and stirred at 300 rpm was added 511 grams of Acalase 2.4L (about 425 mL) (Novozymes North America Inc.). When the temperature of the mixture reached 39°C, the pH was adjusted to 8.0 by the addition of a 50% NaOH in water. A solution of the racemic *N*-Boc-
- 15 (1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester (85g) in 850 mL of DMSO was then added over a period of 40 min. The reaction temperature was then maintained at 40°C for 24.5h during which time the pH of the mixture was adjusted to 8.0 at the 1.5h and 19.5h time points using 50% NaOH in water. After 24.5h, the enantio-excess of the ester was determined to be 97.2%, and the reaction
- 20 was cooled to room temperature (26°C) and stirred overnight (16h) after which the enantio-excess of the ester was determined to be 100%. The pH of the reaction mixture was then adjusted to 8.5 with 50% NaOH and the resulting mixture was extracted with MTBE (2 x 2 L). The combined MTBE extract was then washed with 5% NaHCO₃ (3 x 100 mL), water (3 x 100 mL), and evaporated *in vacuo* to give the
- 25 enantiomerically pure *N*-Boc-(1*R*,2*S*)-1-amino-2-vinylcyclopropane carboxylic acid

ethyl ester as light yellow solid (42.55 g; purity: 97% @ 210 nm, containing no acid; 100% enantiomeric excess ("ee").

The aqueous layer from the extraction process was then acidified to pH 2 with 50% H₂SO₄ and extracted with MTBE (2 x 2 L). The MTBE extract was washed with water (3 x 100 mL) and evaporated to give the acid as light yellow solid (42.74 g; purity: 99% @ 210 nm, containing no ester).



1R, 2S-ester



1S, 2R-acid

| | ester | acid | | |
|---|--|--|-------------------------|-------------|
| High Resolution Mass Spec | (+) ESI, C13H22NO4, [M+H] ⁺ , cal. 256.1549, found 256.1542 | (-) ESI, C11H16NO4, [M-H] ⁻ , cal. 226.1079, found 226.1089 | | |
| NMR observed chemical shift | | | | |
| Solvent: CDCl ₃ (proton δ 7.24 ppm, C-13 δ 77.0 ppm) | | | | |
| Bruker DRX-500C: proton 500.032 MHz, carbon 125.746 MHz | | | | |
| Position | Proton (pattern) ppm | C-13 ppm | Proton (pattern) ppm | C-13 ppm |
| 1 | ---- | 40.9 | ---- | 40.7 |
| 2 | 2.10 (q, J = 9.0 Hz) | 34.1 | 2.17 (q, J = 9.0 Hz) | 35.0 |
| 3a | 1.76 (br) | 23.2 | 1.79 (br) | 23.4 |
| 3b | 1.46 (br) | | 1.51, (br) | |
| 4 | ---- | 170.8 | ---- | 175.8 |
| 5 | 5.74 (ddd, J = 9.0, 10.0, 17.0 Hz) | 133.7 | 5.75 (m) | 133.4 |

| | | | | |
|----|-----------------------------|-------|-----------------------|-------|
| 6a | 5.25 (d, J = 17.0 Hz) | 117.6 | 5.28 (d, J = 17.0 Hz) | 118.1 |
| 6b | 5.08 (dd, J = 10.0, 1.5 Hz) | | 5.12 (d, J = 10.5 Hz) | |
| 7 | ---- | 155.8 | ---- | 156.2 |
| 8 | ---- | 80.0 | ---- | 80.6 |
| 9 | 1.43 (s) | 28.3 | 1.43 (s) | 28.3 |
| 10 | 4.16 (m) | 61.3 | ---- | ---- |
| 11 | 1.23 (t, J = 7.5 Hz) | 14.2 | ---- | ---- |

Resolution B

To 0.5 mL 100 mM Heps•Na buffer (pH 8.5) in a well of a 24 well plate (capacity: 10 mL/well), 0.1 mL of Savinase 16.0L (protease from *Bacillus clausii*) (Novozymes North America Inc.) and a solution of the racemic *N*-Boc-(1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester (10 mg) in 0.1 mL of DMSO were added. The plate was sealed and incubated at 250 rpm at 40°C. After 18h, enantio-excess of the ester was determined to be 44.3% as following: 0.1 mL of the reaction mixture was removed and mixed well with 1 mL ethanol; after centrifugation, 10 microliter ("μl") of the supernatant was analyzed with the chiral HPLC. To the remaining reaction mixture, 0.1 mL of DMSO was added, and the plate was incubated for additional 3 days at 250 rpm at 40°C, after which four mL of ethanol was added to the well. After centrifugation, 10 μl of the supernatant was analyzed with the chiral HPLC and enantio-excess of the ester was determined to be 100%.

Resolution C

To 0.5 ml 100 mM Heps•Na buffer (pH 8.5) in a well of a 24 well plate (capacity: 10 mL/well), 0.1 ml of Esperase 8.0L, (protease from *Bacillus halodurans*) (Novozymes North America Inc.) and a solution of the racemic *N*-Boc-(1*R*,2*S*)/(1*S*,2*R*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester (10 mg) in

0.1 mL of DMSO were added. The plate was sealed and incubated at 250 rpm at 40°C. After 18 hour, enantio-excess of the ester was determined to be 39.6% as following: 0.1 mL of the reaction mixture was removed and mixed well with 1 mL ethanol; after centrifugation, 10 µl of the supernatant was analyzed with the chiral HPLC. To the remaining reaction mixture, 0.1 mL of DMSO was added, and the plate was incubated for additional 3 days at 250 rpm at 40°C, after which four mL of ethanol was added to the well. After centrifugation, 10 µl of the supernatant was analyzed with the chiral HPLC and enantio-excess of the ester was determined to be 100%.

10

Samples analysis was carried out in the following manner:

1) Sample preparation: About 0.5 ml of the reaction mixture was mixed well with 10 volume of EtOH. After centrifugation, 10 µl of the supernatant was injected onto HPLC column.

15

2) Conversion determination:

Column: YMC ODS A, 4.6 x 50 mm, S-5 µm

Solvent: A, 1 millimolar ("mM") HCl in water; B, MeCN

20 Gradient: 30% B for 1 min; 30% to 45% B over 0.5 min; 45% B for 1.5 min; 45% to 30% B over 0.5 min.

Flow rate: 2 mL/min

UV Detection: 210 nm

Retention time: acid, 1.2 min; ester, 2.8 min.

25

3) Enantio-excess determination for the ester:

Column: CHIRACEL OD-RH, 4.6 x 150 mm, S-5 µm

Mobile phase: MeCN/50 mM HClO₄ in water (67/33)

Flow rate: 0.75 mL/min.

30 UV Detection: 210 nm.

Retention time:

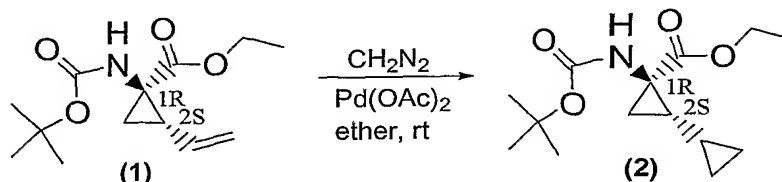
(1S, 2R) isomer as acid: 5.2 min;

Rcaemate: 18.5 min and 20.0 min;

(1R, 2S) isomer as ester: 18.5 min.

II. Preparation of *N*-Boc-(1R,2S)-1-amino-2-cyclopropylcyclopropane carboxylic acid ethyl ester

5



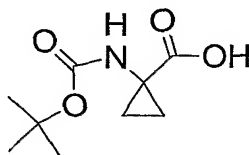
A solution of *N*-Boc-(1R,2S)-1-amino-2-vinylcyclopropane carboxylic acid (255 mg, 1.0 mmol) in ether (10 mL) was treated with palladium acetate (5 mg, 0.022 mmol). The orange/red solution was placed under an atmosphere of N_2 . An excess of

10 diazomethane in ether was added dropwise over the course of 1 h. The resulting solution was stirred at rt for 18 h. The excess diazomethane was removed using a stream of nitrogen. The resulting solution was concentrated by rotary evaporation to give the crude product. Flash chromatography (10% EtOAc/hexane) provided 210

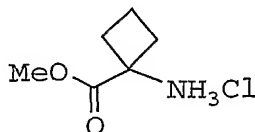
15 mg (78%) of *N*-Boc-(1R,2S)-1-amino-2-cyclopropylcyclopropane carboxylic acid ethyl ester as a colorless oil. LC-MS (retention time: 2.13, similar to method A except: gradient time 3 min, Xterra MS C18 S7 3.0 x 50mm column), MS m/e 270 ($\text{M}^+ + 1$).

III. 1-tert-butoxycarbonylamino-cyclopropane-carboxylic acid is commercially available

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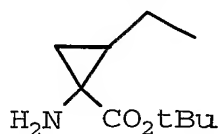


IV. Preparation of 1-aminocyclobutanecarboxylic acid methyl ester·hydrochloride



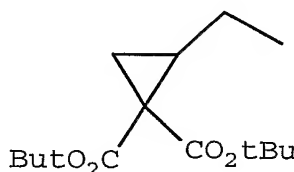
1-aminocyclobutanecarboxylic acid (100 mg, 0.869 mmol)(Tocris) was dissolved in 10 mL of MeOH, HCl gas was bubbled in for 2h. The reaction mixture was stirred for 18 h, and then concentrated in vacuo to give 144 mg of a yellow oil. Trituration with 10 mL of ether provided 100 mg of the titled product as a white solid. ¹H NMR (CDCl₃) δ 2.10-2.25 (m, 1H), 2.28-2.42 (m, 1H), 2.64-2.82 (m, 4H), 3.87 (s, 3H), 9.21 (br s, 3H).

V. Preparation of racemic (1R,2R)/(1S,2S) 1-Amino-2-ethylcyclopropanecarboxylic acid *tert*-butyl ester, shown below.



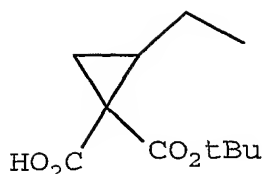
ethyl syn to carboxy

Step 1: Preparation of 2-Ethylcyclopropane-1,1-dicarboxylic acid di-*tert*-butyl ester, shown below.



To a suspension of benzyltriethylammonium chloride (21.0 g, 92.2 mmol) in a 50% aqueous NaOH solution (92.4 g in 185 mL H₂O) was added 1,2-dibromobutane (30.0 g, 138.9 mmol) and di-*tert*-butylmalonate (20.0 g, 92.5 mmol). The reaction mixture was vigorously stirred 18 h at rt, a mixture of ice and water was then added. The crude product was extracted with CH₂Cl₂ (3x) and sequentially washed with water (3x), brine and the organic extracts combined. The organic layer was dried (MgSO₄), filtered and concentrated in vacuo. The resulting residue was flash chromatographed (100 g SiO₂, 3% Et₂O in hexane) to afford the titled product (18.3 g, 67.8 mmol, 73% yield) which was used directly in the next reaction.

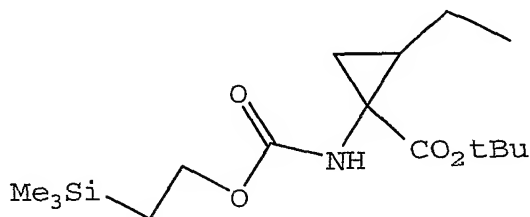
Step 2: Preparation of racemic 2-Ethylcyclopropane-1,1-dicarboxylic acid *tert*-butyl ester, shown below.



The product of Step 1 (18.3 g, 67.8 mmol) was added to a suspension of potassium tert-butoxide (33.55 g, 299.0 mmol) in dry ether (500 mL) at 0 °C, followed by H₂O (1.35 mL, 75.0 mmol) and was vigorously stirred overnight at rt. The reaction mixture was poured in a mixture of ice and water and washed with ether (3x). The aqueous layer was acidified with a 10% aq. citric acid solution at 0°C and extracted with EtOAc (3x). The combined organic layers were washed with water (2x), brine, dried (MgSO₄) and concentrated in vacuo to afford the titled product as a pale yellow oil (10 g, 46.8 mmol, 69% yield).

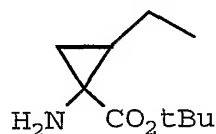
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Step 3: Preparation of (1*R*,2*R*)/(1*S*,2*S*) 2-Ethyl-1-(2-trimethylsilanylethoxycarbonylamino)cyclopropane-carboxylic acid tert-butyl ester, shown below.



- 15 To a suspension, of the product of Step 2 (10 g, 46.8 mmol) and 3 g of freshly activated 4A molecular sieves in dry benzene (160 mL), was added Et₃N (7.50 mL, 53.8 mmol) and DPPA (11 mL, 10.21 mmol). The reaction mixture was refluxed for 3.5 h, 2-trimethylsilyl-ethanol (13.5 mL, 94.2 mmol) was then added, and the reaction mixture was refluxed overnite. The reaction mixture was filtered, diluted with Et₂O, washed with a 10% aqueous citric acid solution, water, saturated aqueous NaHCO₃, water (2x), brine (2X), dried (MgSO₄) and concentrated in vacuo. The residue was suspended with 10g of Aldrich polyisocyanate scavenger resin in 120 mL of CH₂Cl₂, stirred at rt overnite and filtered to afford the titled product (8 g, 24.3 mmol; 52%) as a pale yellow oil: ¹H NMR (CDCl₃) δ 0.03 (s, 9H), 0.97 (m, 5H), 1.20 (bm, 1H), 1.45 (s, 9H), 1.40-1.70 (m, 4H), 4.16 (m, 2H), 5.30 (bs, 1H).
- 20
- 25

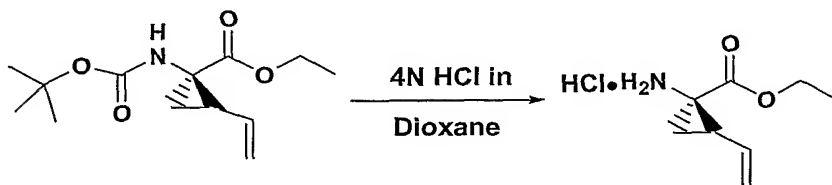
Step 4: Preparation of racemic (1*R*,2*R*)/(1*S*,2*S*) 1-Amino-2-ethylcyclopropanecarboxylic acid *tert*-butyl ester, shown below.



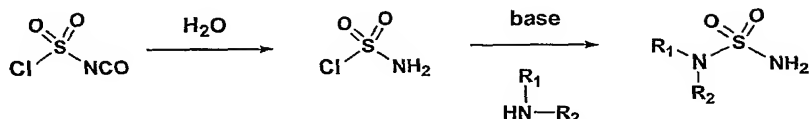
ethyl syn to carboxy

To the product of Step 3 (3 g, 9 mmol) was added a 1.0 M TBAF solution in THF (9.3 mL, 9.3 mmol) and the mixture heated to reflux for 1.5 h, cooled to rt and then diluted with 500 mL of EtOAc. The solution was successively washed with water (2x100 mL), brine (2x100 mL), dried (MgSO₄), concentrated in vacuo to provide the title intermediate

VI. Preparation of chiral (1*R*,2*S*)-1-amino-2-vinylcyclopropane carboxylic acid ethyl ester hydrochloride



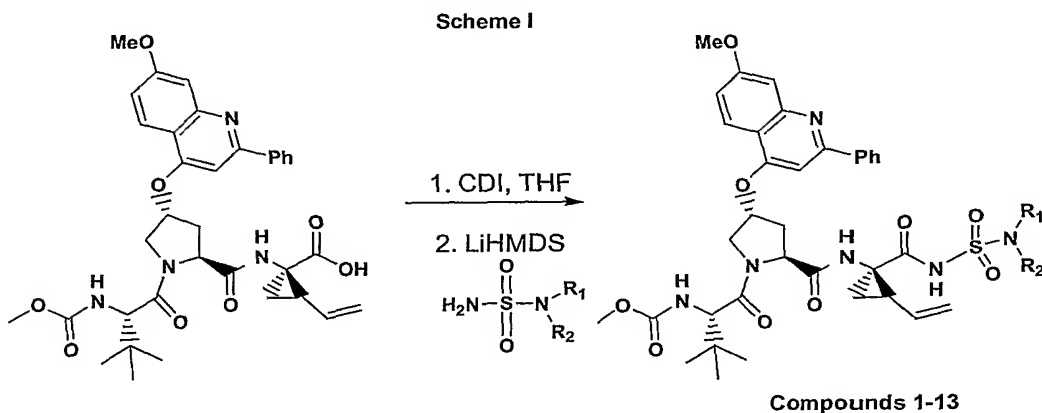
N-BOC (1*R*,2*S*)-1-amino-2-vinylcyclopropanecarboxylic acid ethyl ester (8.5 g, 33.3 mmol) was stirred under an N₂ atmosphere with 200 mL of 4N HCl/dioxane (Aldrich) at rt for 3 h. The solvent was removed under reduced pressure keeping the temperature below 40 C. This gave 6.57 g (~100%) of (1*R*,2*S*)-1-amino-2-vinylcyclopropanecarboxylic acid ethyl ester hydrochloride as a light tan solid. ¹H NMR (300 MHz, CD₃OD) δ 1.31 (t, *J*=7.0 Hz, 3 H), 1.69-1.82 (m, 2 H), 2.38 (q, *J*=8.8 Hz, 1 H), 4.29 (q, *J*=7.0 Hz, 2 H), 5.22 (d, *J*=10.3 Hz, 1 H), 5.40 (d, *J*=17.2 Hz, 1 H), 5.69-5.81 (m, 1 H). LC-MS (Method J, retention time: 0.58 min), MS *m/z* 156 (M⁺+1).

Example 4**General procedure for the preparation of sulfamides**

- 5 The intermediate sulfamoyl chloride was prepared by addition of water (1 equiv) in THF to a cold (-20 °C) stirred solution of chlorosulfonyl isocyanate (1 equiv) in THF and the resulting solution allowed to warm to 0 °C. To this solution was added anhydrous Et₃N (1 equiv) followed by requisite secondary amine (1 equiv). The reaction mixture was warmed to room temperature, then filtered and the filtrate was
- 10 rotary evaporated to afford the desired sulfamides.

Examples 5-18**Preparation of Compounds 1-13.**

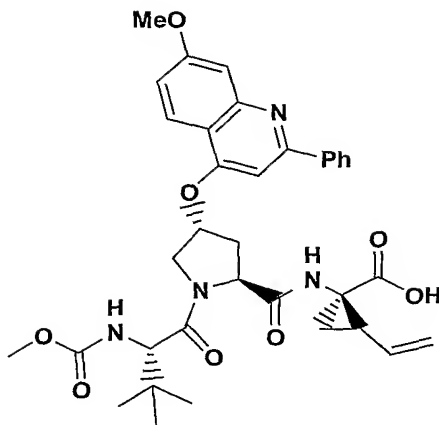
- The key step in the preparation of Compounds 1-13 is the coupling of a tripeptide carboxylic acid and a sulfamide to provide the desired tripeptide sulfamide: The
- 15 general reaction scheme of this key step is shown below:



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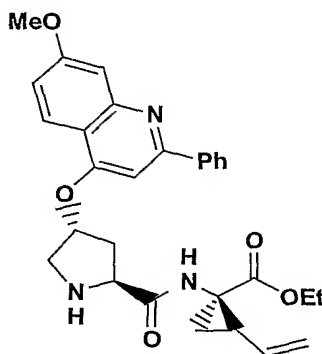
Example 5

Preparation of 1-{[1-(2-Methoxycarbonylamino-3,3-dimethyl-butyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-pyrrolidin-2-carbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid



Step 1 of Example 5

- 5 Preparation of 1-{{4-(7-Methoxy-2-phenyl-quinolin-4-yloxy)-pyrrolidine-2-carbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester

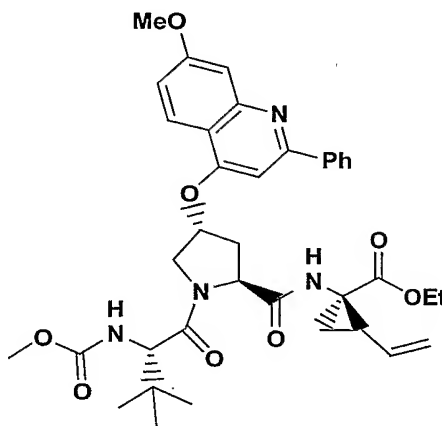


- 10 To a solution of (2S, 4R) 4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester (4.4 g, 9.5 mmol) in CH₃CN (100 mL) was added (1R, 2S) 1-amino-2-vinyl-cyclopropanecarboxylic acid ethyl ester hydrochloride (2.37 g, 12.35 mmol), DIEA (8.27 mL, 47.5 mmol) and the coupling reagent HOBt (2.18 g, 14.25 mmol) and HBTU (5.4 g, 14.25 mmol). The solution was stirred at rt.
- 15 overnight. The solution was then concentrated, washed with water and extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried over MgSO₄ and concentrated to give yellowish oil. It was purified by flash column chromatography (silica gel, 1:1 EtOAc:Hexanes) to give yellowish foam as coupling product. Then it was dissolved in 4N HCl in dioxane (20 mL). The reaction mixture

was stirred at rt. for 4 hr. Then it was concentrated and the crude product was further purified by Prep. HPLC to give yellowish solid as TFA salt. (4.5 g, 77% yield). ¹H NMR (400 MHz, CD₃OD) δ 1.23 (t, *J*=7.09 Hz, 3 H), 1.46 (dd, *J*=9.66, 5.50 Hz, 1 H), 1.78 (dd, *J*=8.07, 5.38 Hz, 1 H), 2.23 (m, 1 H), 2.59 (m, 1 H), 2.95 (m, 1 H), 3.90-3.99 (m, 2 H), 4.05 (s, 3 H), 4.14 (q, *J*=7.09 Hz, 2 H), 4.68 (dd, *J*=10.27, 7.58 Hz, 1 H), 4.86 (m, 1H), 5.12 (dd, *J*=10.27, 1.47 Hz, 1 H), 5.30 (dd, *J*=17.00, 1.34 Hz, 1 H), 5.76 (m, 1 H), 5.96 (s, 1 H), 7.45 (dd, *J*=9.29, 2.45 Hz, 1 H), 7.54 (d, *J*=2.20 Hz, 1 H), 7.62 (s, 1 H), 7.68-7.77 (m, 3 H), 8.05 (m, 2 H), 8.38 (d, *J*=9.29 Hz, 1 H). LC-MS (retention time: 1.243 min.), MS *m/z* 502 (MH⁺).

Step 2 of Example 5

Preparation of 1-{[1-(2-Methoxycarbonylamino-3,3-dimethyl-butyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-pyrrolidine-2-carbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester

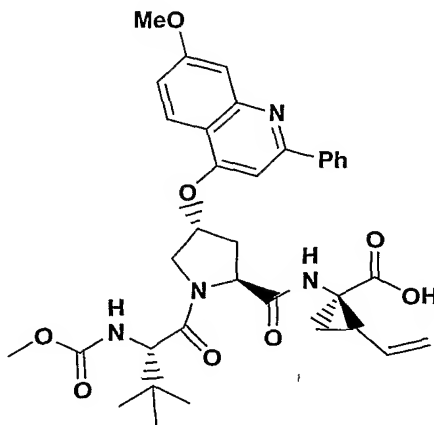


To a solution of 1-{[4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-pyrrolidine-2-carbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid ethyl ester (600 mg, 1.115 mmol) in CH₃CN (20 mL) was 2-methoxycarbonylamino-3,3-dimethyl-butyl acid (317mg, 1.673 mmol), DIEA (0.97 mL, 5.575 mmol) and the coupling reagent HOBt (256 mg, 1.673 mmol) and HBTU (634 g, 1.673 mmol). The solution was stirred at rt. overnight. Then it was concentrated, washed with water and extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried over MgSO₄ and concentrated to give yellowish oil. It was then purified by Prep. HPLC column to give a yellowish oil as product. (410 mg, 47 % yield). ¹H NMR (400 MHz,

CD₃OD) δ 1.03 (s, 9 H), 1.22 (t, $J=7.09$ Hz, 3 H), 1.41 (dd, $J=9.41$, 5.26 Hz, 1 H), 1.71 (dd, $J=8.19$, 5.26 Hz, 1 H), 2.17 (m, 1 H), 2.50 (m, 1 H), 2.77 (m, 1 H), 3.33 (s, 3 H), 4.03 (s, 3 H), 4.04-4.14 (m, 3 H), 4.18 (s, 1 H), 4.61-4.69 (m, 2 H), 4.86 (m, 1H), 5.08 (dd, $J=10.27$, 1.71 Hz, 1 H), 5.25 (d, $J=17.11$ Hz, 1 H), 5.72-5.82 (m, 2 H), 7.39 (dd, $J=9.29$, 2.20 Hz, 1 H), 7.51 (m, 1 H), 7.63 (m, 1 H), 7.69-7.77 (m, 3 H), 8.06 (m, 2 H), 8.35 (d, $J=9.29$ Hz, 1 H), 8.75 (s, 1 H).
 LC-MS (retention time: 2.857 min.), MS m/z 673 (MH^+).

Step 3 of Example 5

- 10 Preparation of 1-{[1-(2-Methoxycarbonylamino-3,3-dimethyl-butyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-pyrrolidine-2-carbonyl]-amino}-2-vinyl-cyclopropanecarboxylic acid



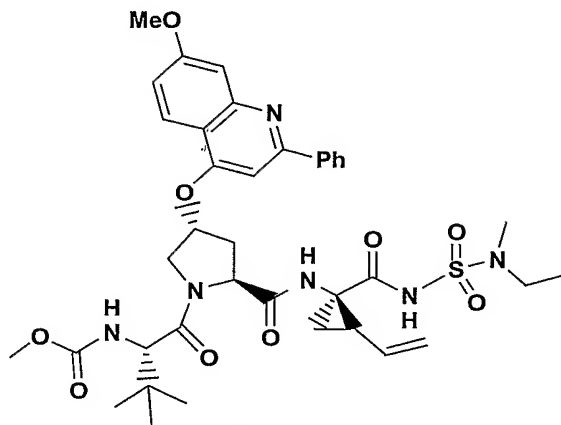
15

- To a solution of 1-{[1-(2-methoxycarbonylamino-3,3-dimethyl-butyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-pyrrolidine-2-carbonyl]-amino}-2-vinyl-cyclopropane-carboxylic acid ethyl ester (410 mg, 0.52 mmol) in THF (20 mL), methanol (11.5 mL) and water (3.8 mL) mixture, lithium hydroxide monohydrate
 20 (328 mg, 7.82 mmol) was added. The reaction mixture was stirred at rt for overnight. Then it was acidified with 1N HCl solution and concentrated. The residue was extracted with EtOAc twice and the organic layers were combined. Then it was dried ($MgSO_4$) and concentrated to give an off-white solid. (230 mg, 69% yield). ¹H NMR (400 MHz, CD₃OD) δ 1.02 (s, 9 H), 1.43 (dd, $J=9.54$, 5.14 Hz, 1 H), 1.68 (dd, $J=8.07$, 5.14 Hz, 1 H), 2.17 (m, 1 H), 2.57 (m, 1 H), 2.75 (m, 1 H), 3.34 (s, 3 H), 4.03
 25

(s, 3 H), 4.06(m, 1H), 4.18 (s, 1 H), 4.60-4.70 (m, 2 H), 4.88 (m, 1H), 5.08 (dd, $J=10.39$, 1.59 Hz, 1 H), 5.25 (dd, $J=17.36$, 1.71 Hz, 1 H), 5.77-5.87 (m, 2 H) 7.38 (dd, $J=9.29$, 2.20 Hz, 1 H), 7.50 (m, 1 H), 7.62 (m, 1 H), 7.69-7.78 (m, 3 H), 8.03-8.07 (m, 2 H), 8.36 (d, $J=9.20$ Hz, 1 H). LC-MS (retention time: 2.177 min.), MS m/z 645 (MH^+).

Example 6

Preparation of Compound 1:



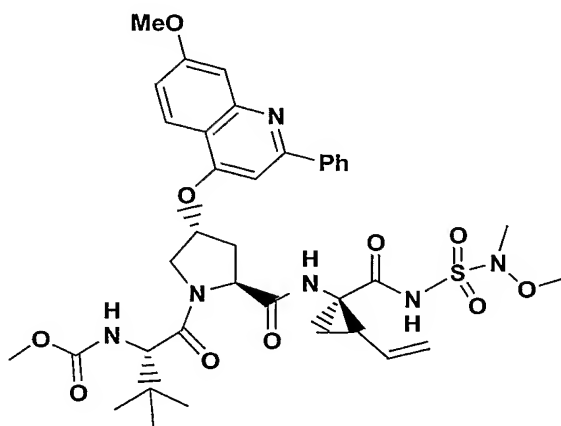
Compound 1

To a solution of 1-[[1-(2-methoxycarbonylamino-3,3-dimethyl-butyl)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-pyrrolidine-2-carbonyl]-amino]-2-vinyl-cyclopropane-carboxylic acid (40 mg, 0.0527 mmol) in THF (5 mL), CDI (12.8 mg, 0.0791 mmol) was added and the reaction mixture was heated under reflux for 45 min. In another round-bottomed flask, LiHMDS (1.0 M solution in hexanes, 0.21 mL, 0.21 mmol) was added to a solution of N-ethylmethanesulfamide (29 mg, 0.21 mmol) in THF (5 mL) and the reaction mixture was stirred at rt for 1h. Two reaction mixtures were added together and stirred at rt for 2h. Water was added to quench the reaction and the reaction solution was extracted with EtOAc. The organic layer was separated and dried over $MgSO_4$. Evaporation of solvent gave crude product which was purified by Prep. HPLC to afford desired N-acylsulfamide. (18.4 mg, 40% yield). 1H NMR (400 MHz, CD_3OD) δ 1.03 (s, 9 H), 1.14(t, $J=7.21$ Hz, 3 H), 1.37 (dd, $J=9.29$, 5.38 Hz, 1 H), 1.84 (dd, $J=8.07$, 5.62 Hz, 1 H), 2.17 (dd, $J=17.36$, 8.80

Hz, 1 H), 2.40 (m, 1 H), 2.72 (m, 1 H), 2.87 (s, 3 H), 3.20-3.30 (m, 2H), 3.36 (s, 3 H), 4.04 (s, 3 H), 4.11 (dd, $J=12.35, 2.81$ Hz, 1 H), 4.21 (s, 1 H), 4.53-4.65 (m, 2 H), 4.82 (m, 1 H), 5.11 (dd, $J=10.15, 1.59$ Hz, 1 H), 5.26 (d, $J=16.87$ Hz, 1 H), 5.70 (m, 1 H), 5.84 (s, br, 1 H), 7.41 (dd, $J=9.29, 2.20$ Hz, 1 H), 7.52 (d, $J=2.20$ Hz, 1 H), 7.64 (s, 1 H) 7.68-7.80 (m, 3 H), 8.03-8.10 (m, 2 H), 8.34 (d, $J=9.29$ Hz, 1 H), 9.16 (s, 1 H).
LC-MS (retention time: 2.333 min.), MS m/z 765 (MH^+).

Example 7

Preparation of Compound 2



Compound 2

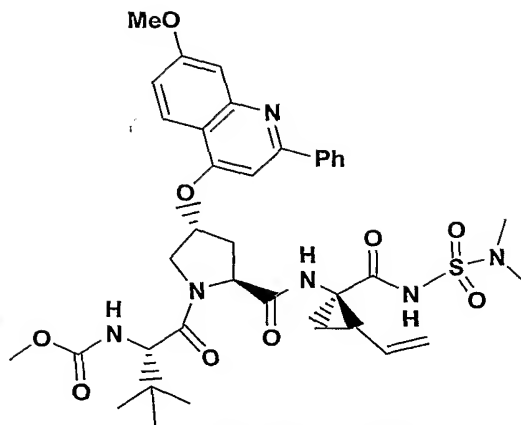
10

Compound 2 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 2 however, the acylsulfamide employed was synthesized using methoxymethylamine in place of methylethylamine. LC-MS (retention time: 2.070 min.), MS m/z 767 (MH^+).

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Example 8

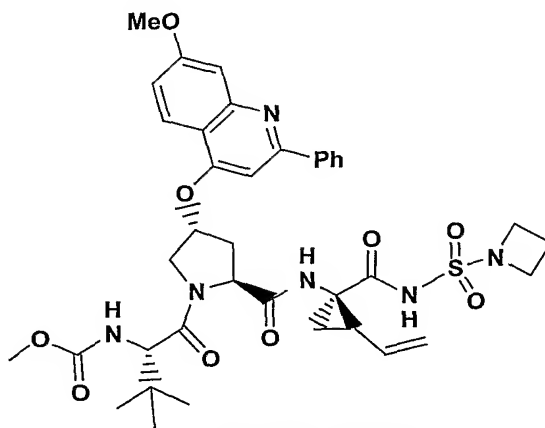
Preparation of Compound 3:

**Compound 3**

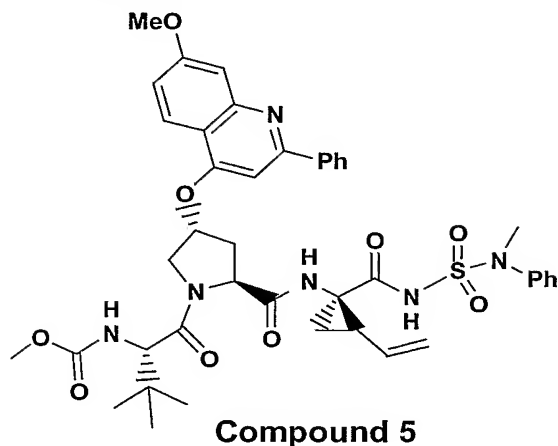
Compound 3 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 3 however, the acylsulfamide employed was synthesized using dimethylamine in place of methylethylamine. LC-MS (retention time: 2.243 min.), MS m/z 751 (MH^+).

Example 9

Preparation of Compound 4:

**Compound 4**

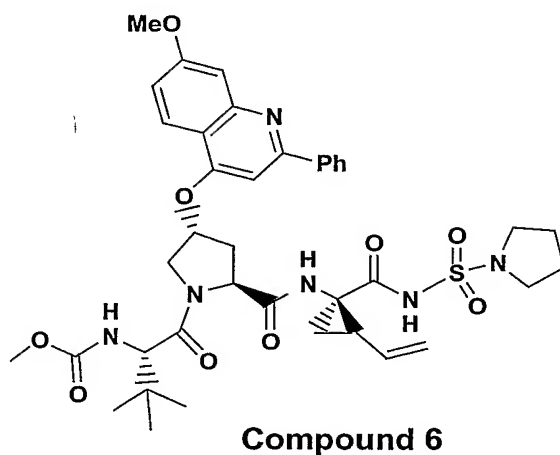
Compound 4 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 4 however, the acylsulfamide employed was synthesized using azetidine in place of methylethylamine. LC-MS (retention time: 2.223 min.), MS m/z 763 (MH^+).

Example 10**Preparation of Compound 5:**

5

Compound 5 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 5 however, the acylsulfamide employed was synthesized using N-methylaniline in place of methylethylamine. LC-MS (retention time: 2.503 min.), MS m/z 813 (MH⁺).

10

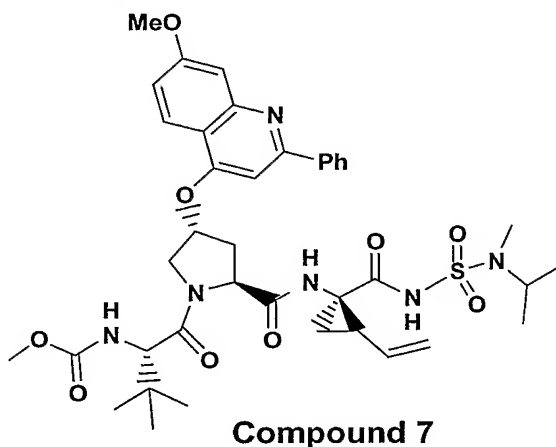
Example 11**Preparation of Compound 6:**

15

Compound 6 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 6 however, the

acysulfamide employed was synthesized using pyrrolidine place of methylethylamine ^1H NMR(400 MHz, CD_3OD) δ 1.02(s, 9 H), 1.37 (dd, $J=9.3$ Hz, 5.1 Hz, 1 H), 1.82-1.90 (m, 5 H), 2.19 (m, 1 H), 2.40 (m, 1 H), 2.74 (m, 1 H), 3.31-3.49 (m, 7 H), 4.04 (s, 3 H), 4.11 (m, 1 H), 4.21 (s, 1 H), 4.54-4.64 (m, 2 H), 4.85 (m, 1 H), 5.11(d, $J=10.3$ Hz, 1 H), 5.28 (d, $J=17.1$ Hz, 1 H), 5.69 (m, 1 H), 5.84 (s, br, 1 H), 7.41 (dd, $J=9.3$ Hz, 1.9 Hz, 1 H), 7.52 (d, $J=2.4$ Hz, 1 H), 7.63 (s, 1 H), 7.68-7.77 (m, 3 H), 8.06 (m, 2 H), 8.34 (d, $J=9.3$ Hz, 1 H). LC-MS (retention time: 2.340 min.), MS m/z 777 (MH^+).

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Example 12**Preparation of Compound 7**

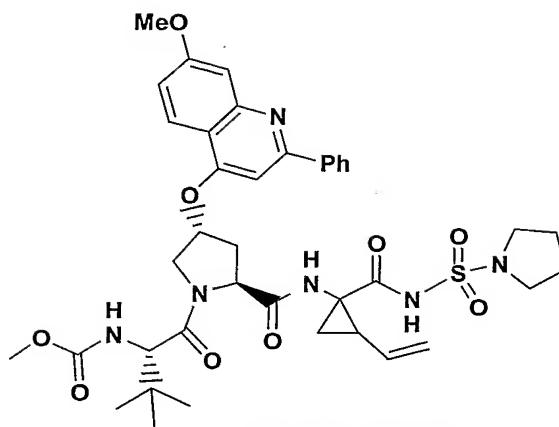
15

Compound 7 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 7 however, the acysulfamide employed was synthesized using isopropylmethylaniline in place of methylethylamine. LC-MS (retention time: 2.420 min.), MS m/z 779 (MH^+).

Example 13

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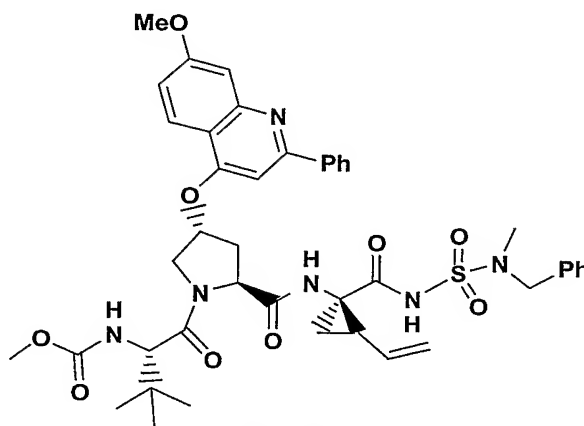
Preparation of Compound 8

**Compound 8**

Compound 2 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 8 however, the acylsulfamide employed was synthesized using pyrrolidine in place of methylethylamine and a racemic P1 element. MS m/z 777 (MH^+).

Example 14

Preparation of Compound 9

**Compound 9**

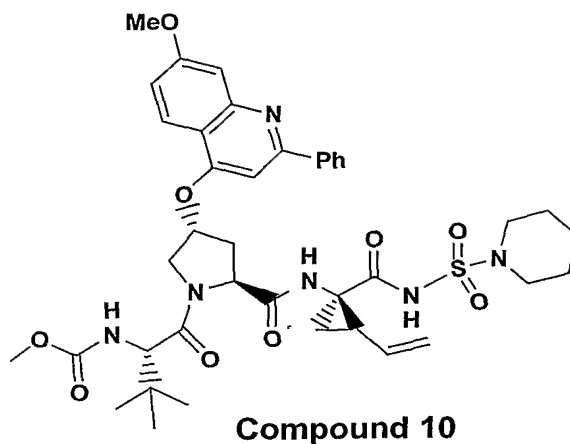
10

Compound 2 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 9 however, the acylsulfamide employed was synthesized using methylbenzylamine in place of methylethylamine. LC-MS (retention time: 2.430 min.), MS m/z 827 (MH^+).

15

Example 15

Preparation of Compound 10



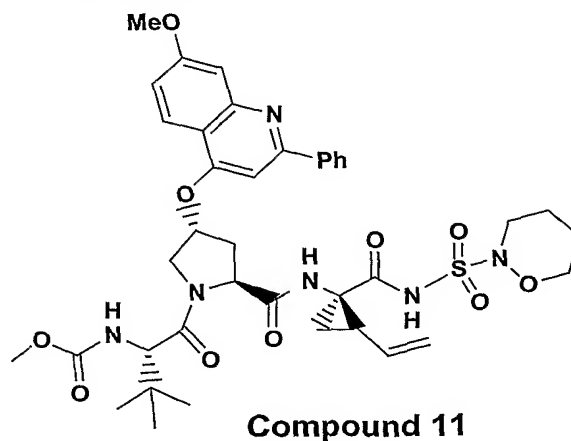
5

Compound 10 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 10 however, the acylsulfamide employed was synthesized using piperidine in place of methylethylamine. LC-MS (retention time: 2.453 min.), MS m/z 791 (MH^+).

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Example 16

Preparation of Compound 11



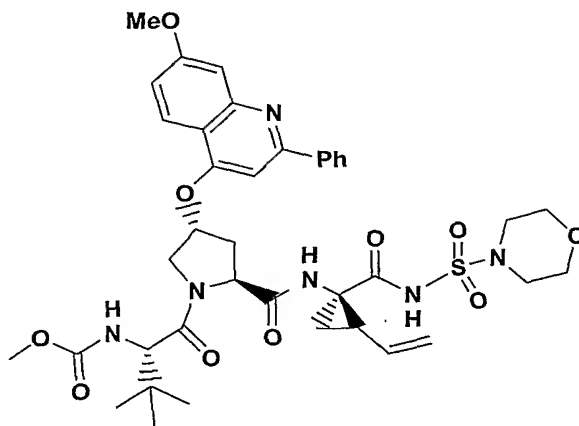
15

Compound 11 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 11 however, the

acylsulfamide employed was synthesized using tetrahydro-2H-1,2-oxazine in place of methylethylamine. LC-MS (retention time: 2.317 min.), MS m/z 793 (MH^+).

Example 17

5 Preparation of Compound 12



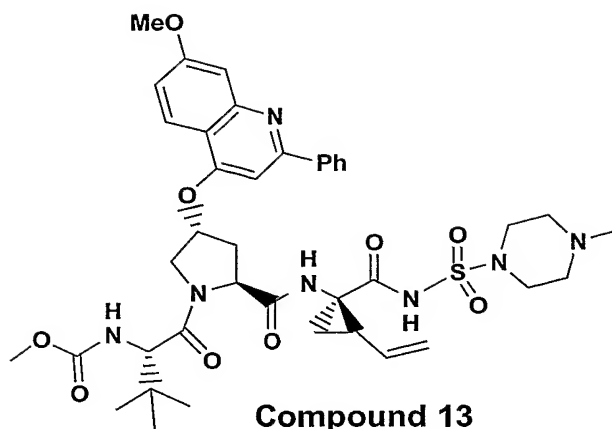
Compound 12

Compound 12 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 12 however, the acylsulfamide employed was synthesized using morpholine in place of methylethylamine. 1H NMR(400 MHz, CD_3OD) δ 1.02(s, 9 H), 1.38 (dd, $J=9.1$ Hz, 5.6 Hz, 1 H), 1.88 (dd, $J=8.1$ Hz, 5.6 Hz, 1 H), 2.21 (m, 1 H), 2.40 (m, 1 H), 2.75 (m, 1 H), 3.36 (s, 3 H), 3.66 (m, 4 H), 4.04 (s, 3 H), 4.10 (dd, $J=12.5$ Hz, 2.9 Hz, 1 H), 4.21 (s, 1 H), 4.55-4.65 (m, 2 H), 4.85 (m, 5 H), 5.15(dd, $J=10.3$ Hz, 1.5 Hz, 1 H), 5.29 (dd, $J=17.1$ Hz, 1.5 Hz, 1 H), 5.70 (m, 1 H), 5.83 (s, br, 1 H), 7.41 (dd, $J=9.3$ Hz, 2.2 Hz, 1 H), 7.52 (d, $J=2.2$ Hz, 1 H), 7.63 (s, 1 H), 7.69-7.79 (m, 3 H), 8.07 (m, 2 H), 8.34 (d, $J=9.3$ Hz, 1 H). LC-MS (retention time: 2.210 min.), MS m/z 793 (MH^+).

20

Example 18

Preparation of Compound 13



Compound 13 was prepared by the route shown in Scheme 1 of Example 5 and as described for Compound 1 of Example 6. In the case of Compound 13 however, the acylsulfamide employed was synthesized using N-methylpiperizine in place of

5 methylethylamine. LC-MS (retention time: 1.923 min.), MS m/z 806 (MH^+).

Examples 19-27

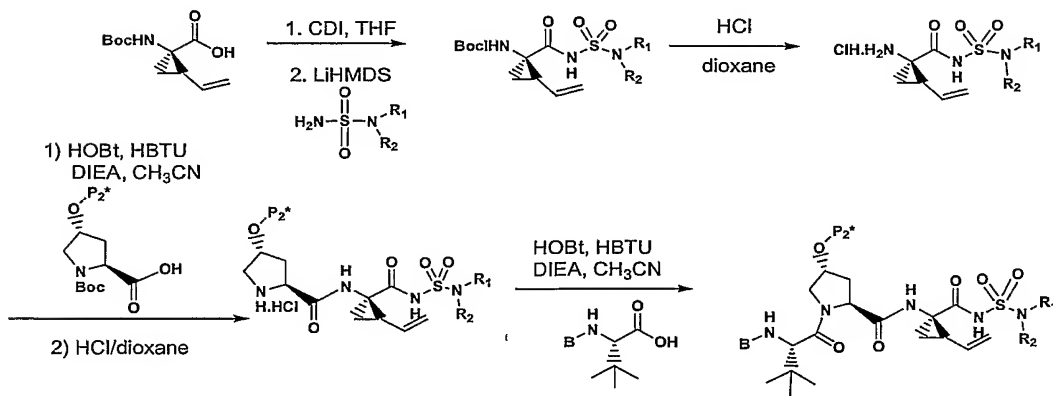
Preparation of Compounds 14-22.

10

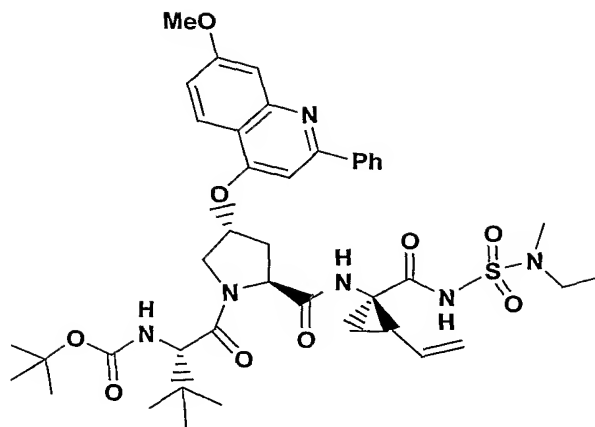
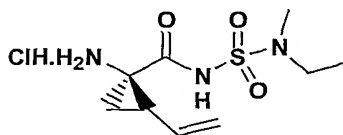
Example 19

General scheme for the preparation of Compounds 14-22

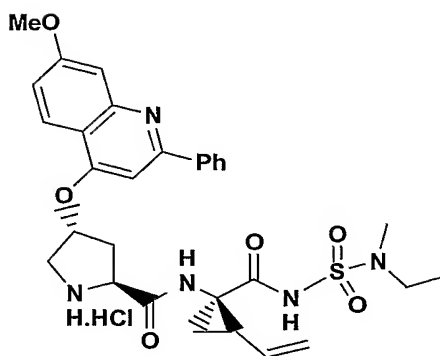
Scheme 2



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**Compound 14****Step 1 of Example 19**

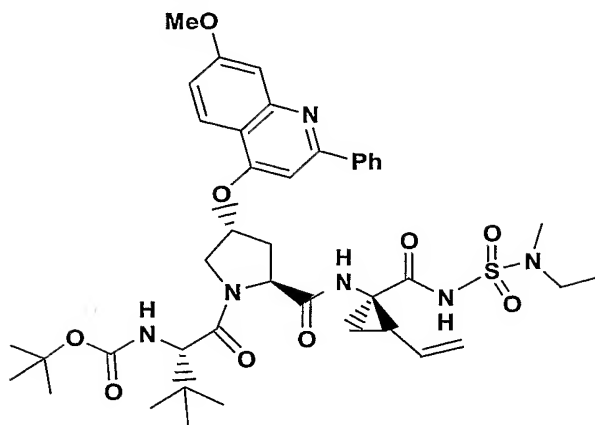
- 5 To a solution of (1R, 2S) 1-tert-butoxycarbonylamino-2-vinyl-cyclopropanecarboxylic acid (217 mg, 1.194 mmol) in THF (5 mL), CDI (290 mg, 1.791 mmol) was added and the reaction mixture was heated under reflux for 45 min. In another round-bottomed flask, LiHMDS (1.0 M solution in hexanes, 2.4 mL, 2.4 mmol) was added to a solution of N-ethylmethylsulfamide (330 mg, 2.388 mmol) in
- 10 THF (5 mL) and the reaction mixture was stirred at rt for 1h. Two reaction mixtures were added together and stirred at rt for 2h. Water was added to quench the reaction and the reaction solution was extracted with EtOAc. The organic layer was separated and dried over MgSO₄. Evaporation of solvent gave crude product which was purified by Prep. HPLC to afford desired N-acylsulfamide. N-acylsulfamide was
- 15 then dissolved in 4N HCl solution in dioxane (2mL) and stirred at rt for 4h. Evaporation of solution give brownish oil as HCl salt. (112mg, 33% yield). ¹H NMR (400Mz, CD₃OD) δ 1.16 (t, *J*=7.21 Hz, 3 H), 1.68 (dd, *J*=10.03, 7.83 Hz, 1 H), 2.15 (m, 1 H), 2.37 (m, 1 H), 2.89 (s, 3 H), 3.30 (m, 2 H), 5.31 (d, *J*=10.27 Hz, 1 H), 5.42 (d, *J*=17.12 Hz, 3 H), 5.68 (m, 1 H).
- 20 LC-MS (retention time: 0.883 min.), MS *m/z* 270 (M+Na⁺).

Step 2 of Example 19

To a solution of (2S, 4R)-4-(7-methoxy-2-phenyl-quinolin-4-yloxy)-pyrrolidine-1,2-dicarboxylic acid 1-tert-butyl ester (62.5 mg, 0.135 mmol) in CH₃CN (10 mL) was added the acylsulfamide-P1-amino acid (42 mg, 0.148 mmol), DIEA (0.12 mL, 0.675 mmol) and the coupling reagent HOBt (31 mg, 0.203 mmol) and HBTU (77 mg, 0.203 mmol). The solution was stirred at rt for overnight. Then it was concentrated, washed with water and extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried over MgSO₄ and concentrated to give yellow oil. It was purified by Prep. HPLC column to give a colorless thick oil which was then dissolved in 4N HCl in dioxane (2 mL). The reaction mixture was stirred at rt. for overnight. Evaporation of solvent gave the desired product as a hydrochloride salt (62 mg, yellowish oil, 68% yield). ¹H NMR (400 MHz, CD₃OD) δ 1.14 (t, *J*=7.21 Hz, 3 H), 1.32 (dd, *J*=9.78, 5.62 Hz, 1 H), 1.90 (dd, *J*=7.58, 5.62 Hz, 1 H), 2.31 (m, 1 H), 2.51 (m, 1 H), 2.86 (s, 3 H), 3.06 (m, 1 H), 3.20-3.30(m, 2 H), 3.56 (m, 1 H), 3.72 (m, 2 H), 3.96 (m, 1 H), 4.06 (s, 3 H), 4.80(m, 1H), 5.13 (d, *J*=10.52 Hz, 1 H), 5.29 (d, *J*=16.87 Hz, 1 H), 5.60 (m, 1 H), 5.97 (s, 1 H), 7.49 (dd, *J*=9.29, 1.96 Hz, 1 H), 7.57 (d, *J*=1.96 Hz, 1 H), 7.63 (s, 1 H), 7.68-7.79 (m, 3 H), 8.08 (m, 2 H), 8.50 (d, *J*=9.29 Hz, 1 H). LC-MS (retention time: 1.810 min.), MS *m/z* 594 (MH⁺).

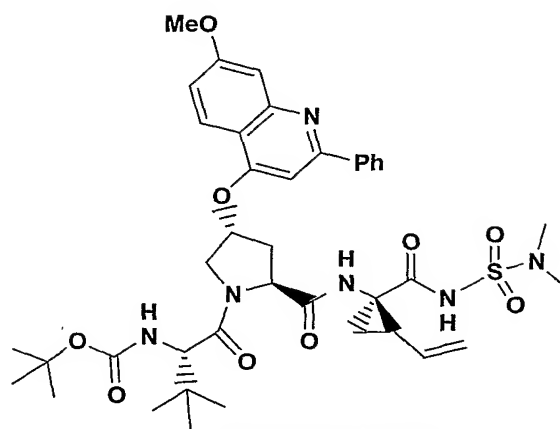
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Step 3 of Example 19**Compound 14**

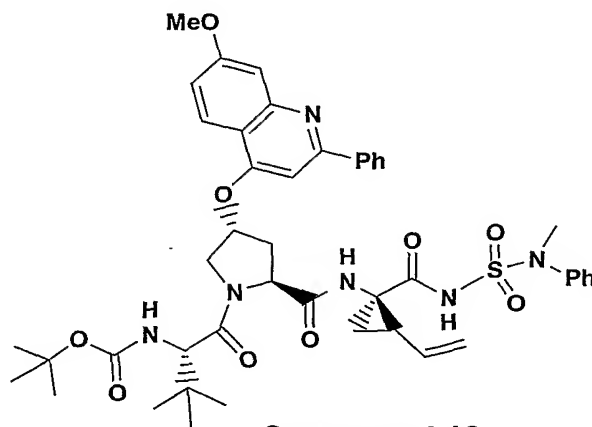
To a solution of dipeptide sulfamide (product of step 2 of Example 19, 14 mg, 0.021 mmol) in CH₃CN (5 mL) was added N-boc-L-t-leucine (7.3 mg, 0.0315 mmol), DIEA (0.022 mL, 0.126 mmol) and the coupling reagent HOBt (4.8 mg, 0.0315 mmol) and HBTU (12 mg, 0.0315 mmol). The solution was stirred at rt for overnight. Then it was concentrated, washed with water and extracted with ethyl acetate twice. The combined organic layers were washed with brine, dried over MgSO₄ and concentrated to give yellow oil. It was purified by Prep. HPLC column to give a white solid as final product. (9.0 mg, 53% yield). ¹H NMR (400 MHz, CD₃OD) δ 1.02 (s, 9 H), 1.13 (t, *J*=7.09 Hz, 3 H), 1.25 (s, 9 H), 1.37 (m, 1 H), 1.82 (m, 1 H), 2.15 (m, 1 H), 2.30 (m, 1 H), 2.64 (m, 1 H), 2.87 (s, 3 H), 3.20-3.30 (m, 2 H), 3.94 (s, 3H), 4.07 (m, 1 H), 4.25 (m, 1 H), 4.47-4.63 (m, 3 H), 5.09 (d, *J*=10.51 Hz, 1 H), 5.25 (d, *J*=17.37 Hz, 1 H), 5.56 (s, br, 1 H), 5.71 (m, 1 H), 6.66 (d, *J*=9.05 Hz, 1 H), 7.07 (m, 1 H), 7.24 (s, 1 H), 7.38 (d, *J*=1.96 Hz, 1 H), 7.46-7.56 (m, 3 H), 8.01-8.09 (m, 3 H). LC-MS (retention time: 2.563 min.), MS *m/z* 807 (MH⁺).

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Example 20**Preparation of Compound 15****Compound 15**

Compound 15 was prepared by the route shown in Scheme 2 of Example 19 and as described for Compound 14 of Example 19. In the case of Compound 15 however, the acylsulfamide employed was synthesized using dimethylamine in place of methylethylamine.. LC-MS (retention time: 2.463 min.), MS m/z 793 (MH⁺).

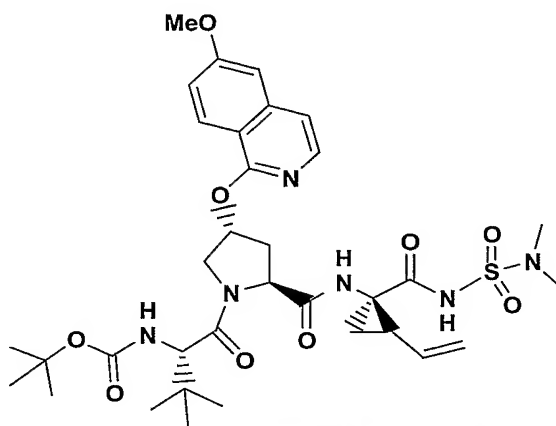
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Example 21**Preparation of Compound 16****Compound 16**

Compound 16 was prepared by the route shown in Scheme 2 of Example 19 and as described for Compound 14 of Example 19. In the case of Compound 16 however, the acylsulfamide employed was synthesized using N-methylaniline in place of methylethylamine. LC-MS (retention time: 2.723 min.), MS m/z 856 (MH⁺).

Example 22

Preparation of Compound 17



Compound 17

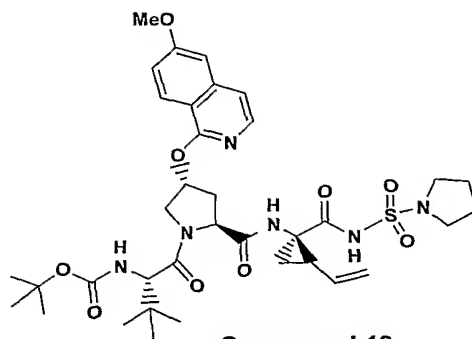
10

Compound 17 was prepared by the route shown in Scheme 2 of Example 19 and as described for Compound 14 of Example 19. In the case of Compound 17 however, the acylsulfamide employed was synthesized using dimethylamine in place of methylethylamine, and 6-methoxy-1-chloroisoquinoline was used in place of 2-phenyl-4-chloro-7-methoxyquinoline. LC-MS (retention time: 2.807 min.), MS m/z 717 (MH⁺).

15

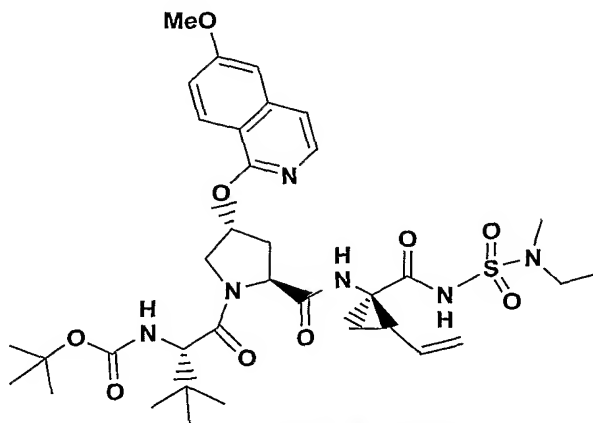
Example 23

Preparation of Compound 18

**Compound 18**

Compound 18 was prepared by the route shown in Scheme 2 of Example 19 and as described for Compound 14 of Example 19. In the case of Compound 18 however, the acylsulfamide employed was synthesized using pyrrolidine in place of
 5 methylethylamine, and 6-methoxy-1-chloroisoquinoline was used in place of 2-phenyl-4-chloro-7-methoxyquinoline. LC-MS (retention time: 2.927 min.), MS m/z 743 (MH^+).

10

Example 24**Preparation of Compound 19****Compound 19**

15 Compound 19 was prepared by the route shown in Scheme 2 of Example 19 and as described for Compound 14 of Example 19. In the case of Compound 19 however, 6-methoxy-1-chloroisoquinoline was used in place of 2-phenyl-4-chloro-7-methoxyquinoline. 1H NMR (400 MHz, CD_3OD) δ 1.02 (s, 9 H), 1.14 (t, $J=7.21$ Hz,

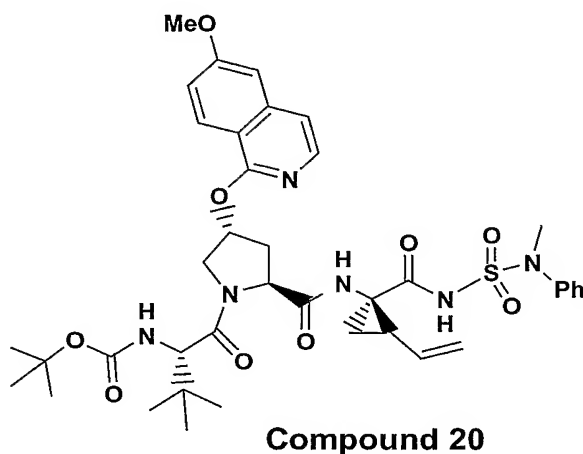
3 H), 1.25 (s, 9 H), 1.37 (m, 1 H), 1.81 (m, 1 H), 2.13-2.30(m, 2 H), 2.57 (m, 1 H), 2.87 (s, 3 H), 3.20-3.30(m, 2 H), 3.90 (s, 3 H), 4.04(m, 1 H), 4.23 (m, 1 H), 4.42 (d, $J=12.22$ Hz, 1 H), 4.46-4.60(m, 2H), 5.11 (d, $J=10.27$ Hz, 1 H), 5.29 (d, $J=16.87$ Hz, 1 H), 5.70 (m, 1 H), 5.81 (s, br, 1 H), 6.60 (d, $J=8.56$ Hz, 1 H), 7.08 (d, $J=8.07$ Hz, 1 H), 7.16 (d, $J=2.45$ Hz, 1 H), 7.23 (d, $J=5.87$ Hz, 1 H), 7.87 (d, $J=5.87$ Hz, 1 H), 8.06 (d, $J=9.05$ Hz, 1 H).

LC-MS (retention time: 2.910 min.), MS m/z 731 (MH^+).

10

Example 25

Preparation of Compound 20



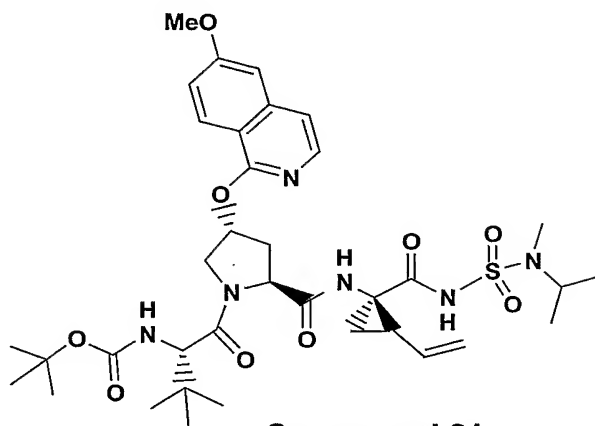
15 Compound 20 was prepared by the route shown in Scheme 2 of Example 19 and as described for Compound 14 of Example 19. In the case of Compound 20 however, the acylsulfamide employed was synthesized using N-methylaniline in place of methylethylamine, and 6-methoxy-1-chloroisoquinoline was used in place of 2-phenyl-4-chloro-7-methoxyquinoline. 1H NMR (400 MHz, CD_3OD) δ 0.95 (s, 9 H),
20 1.26 (s, 9 H), 1.41 (m, 1 H), 1.87 (m, 1 H), 2.12-2.25 (m, 2 H), 2.50 (dd, $J=13.94$, 7.09 Hz, 1 H), 3.39 (s, 3 H), 3.89 (s, 3 H), 4.02 (m, 1 H), 4.19 (d, $J=9.29$ Hz, 1 H), 4.31-4.47 (m, 2 H), 4.57 (s, 1 H), 5.17 (d, $J=10.27$ Hz, 1 H), 5.31 (d, $J=16.63$ Hz, 1 H), 5.73-5.90 (m, 2 H), 6.56 (d, $J=9.29$ Hz, 1 H), 7.04 (d, $J=8.56$ Hz, 1 H), 7.15 (d, $J=1.96$ Hz, 1 H), 7.22 (d, $J=5.87$ Hz, 1 H), 7.24-7.40 (m, 5 H), 7.86 (d, $J=5.87$ Hz, 1
25 H), 8.03 (d, $J=9.05$ Hz, 1 H).

LC-MS (retention time: 3.017 min.), MS m/z 779 (MH^+).

Example 26

Preparation of Compound 21

5



Compound 21

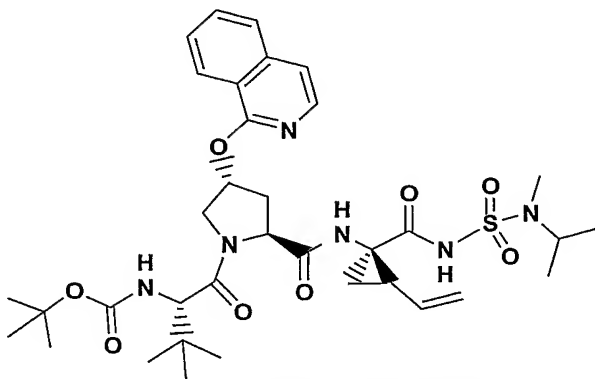
Compound 21 was prepared by the route shown in Scheme 2 of Example 19 and as described for Compound 14 of Example 19. In the case of Compound 21 however, the acylsulfamide employed was synthesized using methylisopropylamine in place of methylethylamine, and 6-methoxy-1-chloroisoquinoline was used in place of 2-phenyl-4-chloro-7-methoxyquinoline. LC-MS (retention time: 2.963 min.), MS m/z 768 ($M+Na^+$).

10

Example 27

Preparation of Compound 22

15

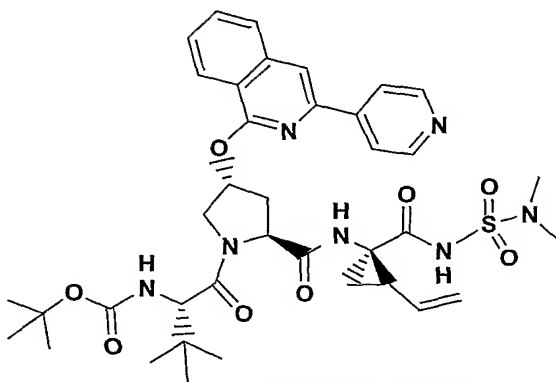


Compound 22

Compound 22 was prepared by the route shown in Scheme 2 of Example 19 and as described for Compound 14 of Example 19. In the case of Compound 22 however, the acylsulfamide employed was synthesized using methylisopropylamine in place of methylethylamine, and 1-chloroisoquinoline was used in place of 2-phenyl-4-chloro-7-methoxyquinolin LC-MS (retention time: 2.993 min.), MS m/z 737 (M+Na⁺).

Example 28

Compound 23



Compound 23

Compound 23 can be prepared by synthesis methods such as described herein.

Example 29

Biological Studies

Recombinant HCV NS3/4A protease complex FRET peptide assay

The purpose of this in vitro assay was to measure the inhibition of HCV NS3 protease complexes, derived from the BMS strain, H77 strain or J416S strain, as described below, by compounds of the present invention. This assay provides an indication of how effective compounds of the present invention would be in inhibiting HCV proteolytic activity.

Serum from an HCV-infected patient was obtained from Dr. T. Wright, San Francisco Hospital. An engineered full-length cDNA (compliment deoxyribonucleic

acid) template of the HCV genome (BMS strain) was constructed from DNA fragments obtained by reverse transcription-PCR (RT-PCR) of serum RNA (ribonucleic acid) and using primers selected on the basis of homology between other genotype 1a strains. From the determination of the entire genome sequence, a
5 genotype 1a was assigned to the HCV isolate according to the classification of Simmonds et al. (See P Simmonds, KA Rose, S Graham, SW Chan, F McOmish, BC Dow, EA Follett, PL Yap and H Marsden, J. Clin. Microbiol., 31(6), 1493-1503 (1993)). The amino acid sequence of the nonstructural region, NS2-5B, was shown to be >97% identical to HCV genotype 1a (H77) and 87% identical to genotype 1b
10 (J4L6S). The infectious clones, H77 (1a genotype) and J4L6S (1b genotype) were obtained from R. Purcell (NIH) and the sequences are published in Genbank (AAB67036, see Yanagi, M., Purcell, R.H., Emerson, S.U. and Bukh, J. Proc. Natl. Acad. Sci. U.S.A. 94(16), 8738-8743 (1997); AF054247, see Yanagi, M., St Claire, M., Shapiro, M., Emerson, S.U., Purcell, R.H. and Bukh, J. Virology 244 (1), 161-172.
15 (1998)).

The H77 and J4L6S strains were used for production of recombinant NS3/4A protease complexes. DNA encoding the recombinant HCV NS3/4A protease complex (amino acids 1027 to 1711) for these strains were manipulated as described
20 by P. Gallinari et al. (see Gallinari P, Paolini C, Brennan D, Nardi C, Steinkuhler C, De Francesco R. Biochemistry. 38(17):5620-32, (1999)). Briefly, a three-lysine solubilizing tail was added at the 3'-end of the NS4A coding region. The cysteine in the P1 position of the NS4A-NS4B cleavage site (amino acid 1711) was changed to a glycine to avoid the proteolytic cleavage of the lysine tag. Furthermore, a cysteine to
25 serine mutation was introduced by PCR at amino acid position 1454 to prevent the autolytic cleavage in the NS3 helicase domain. The variant DNA fragment was cloned in the pET21b bacterial expression vector (Novagen) and the NS3/4A complex was expressed in *Escherichia coli* strain BL21 (DE3) (Invitrogen) following the protocol described by P. Gallinari et al. (see Gallinari P, Brennan D,
30 Nardi C, Brunetti M, Tomei L, Steinkuhler C, De Francesco R., J Virol. 72(8):6758-69 (1998)) with modifications. Briefly, the NS3/4A protease complex expression was induced with 0.5 millimolar (mM) Isopropyl β -D-1-thiogalactopyranoside (IPTG) for 22 hours (h) at 20°C. A typical fermentation (1 Liter (L)) yielded

approximately 10 grams (g) of wet cell paste. The cells were resuspended in lysis buffer (10 mL/g) consisting of 25 mM N-(2-Hydroxyethyl)Piperazine-N'-(2-Ethane Sulfonic acid) (HEPES), pH 7.5, 20% glycerol, 500 mM Sodium Chloride (NaCl), 0.5% Triton X-100, 1 microgram/milliliter ("µg/mL") lysozyme, 5 mM Magnesium Chloride (MgCl₂), 1 µg/ml DnaseI, 5mM β-Mercaptoethanol (βME), Protease inhibitor - Ethylenediamine Tetraacetic acid (EDTA) free (Roche), homogenized and incubated for 20 minutes (min) at 4°C. The homogenate was sonicated and clarified by ultra-centrifugation at 235000 g for 1 h at 4°C. Imidazole was added to the supernatant to a final concentration of 15 mM and the pH adjusted to 8.0. The crude protein extract was loaded on a Nickel - Nitrilotriacetic acid (Ni-NTA) column pre-equilibrated with buffer B (25 mM HEPES, pH 8.0, 20% glycerol, 500 mM NaCl, 0.5% Triton X-100, 15 mM imidazole, 5 mM βME). The sample was loaded at a flow rate of 1 mL/min. The column was washed with 15 column volumes of buffer C (same as buffer B except with 0.2% Triton X-100). The protein was eluted with 5 column volumes of buffer D (same as buffer C except with 200 mM Imidazole).

NS3/4A protease complex-containing fractions were pooled and loaded on a desalting column Superdex-S200 pre-equilibrated with buffer D (25mM HEPES, pH 7.5, 20% glycerol, 300 mM NaCl, 0.2% Triton X-100, 10 mM βME). Sample was loaded at a flow rate of 1 mL/min. NS3/4A protease complex-containing fractions were pooled and concentrated to approximately 0.5 mg/ml. The purity of the NS3/4A protease complexes, derived from the BMS, H77 and J4L6S strains, were judged to be greater than 90% by SDS-PAGE and mass spectrometry analyses.

The enzyme was stored at -80°C, thawed on ice and diluted prior to use in assay buffer. The substrate used for the NS3/4A protease assay was RET S1 (Resonance Energy Transfer Depsipeptide Substrate; AnaSpec, Inc. cat # 22991)(FRET peptide), described by Taliani et al. in *Anal. Biochem.* 240(2):60-67 (1996). The sequence of this peptide is loosely based on the NS4A/NS4B natural cleavage site for the HCV NS3 protease except there is an ester linkage rather than an amide bond at the cleavage site. The peptide substrate was incubated with one of the three recombinant NS3/4A protease complexes, in the absence or presence of a

compound of the present invention, and the formation of fluorescent reaction product was followed in real time using a Cytofluor Series 4000.

The reagents were as follow: HEPES and Glycerol (Ultrapure) were obtained from GIBCO-BRL. Dimethyl Sulfoxide (DMSO) was obtained from Sigma. β -Mercaptoethanol was obtained from Bio Rad.

Assay buffer: 50 mM HEPES, pH 7.5; 0.15 M NaCl; 0.1% Triton; 15% Glycerol; 10 mM β ME. Substrate: 2 μ M final concentration (from a 2 mM stock solution in DMSO stored at -20°C). HCV NS3/4A protease type 1a (1b), 2-3 nM final concentration (from a 5 μ M stock solution in 25 mM HEPES, pH 7.5, 20% glycerol, 300 mM NaCl, 0.2% Triton-X100, 10 mM β ME). For compounds with potencies approaching the assay limit, the assay was made more sensitive by adding 50 μ g/ml Bovine Serum Albumin (Sigma) to the assay buffer and reducing the end protease concentration to 300 pM.

The assay was performed in a 96-well polystyrene black plate from Falcon. Each well contained 25 μ l NS3/4A protease complex in assay buffer, 50 μ l of a compound of the present invention in 10% DMSO/assay buffer and 25 μ l substrate in assay buffer. A control (no compound) was also prepared on the same assay plate. The enzyme complex was mixed with compound or control solution for 1 min before initiating the enzymatic reaction by the addition of substrate. The assay plate was read immediately using the Cytofluor Series 4000 (Perspective Biosystems). The instrument was set to read an emission of 340 nm and excitation of 490 nm at 25°C. Reactions were generally followed for approximately 15 min.

The percent inhibition was calculated with the following equation:

$$100 - [(\delta F_{\text{inh}}/\delta F_{\text{con}}) \times 100]$$

where δF is the change in fluorescence over the linear range of the curve. A non-linear curve fit was applied to the inhibition-concentration data, and the 50%

effective concentration (IC₅₀) was calculated by the use of Excel Xl-fit software using the equation, $y=A+((B-A)/(1+((C/x)^D)))$.

All of the compounds tested were found to inhibit the activity of the NS3/4A protease complex with IC₅₀'s of 1.2 μ M or less. Further, compounds of the present invention, which were tested against more than one type of NS3/4A complex, were found to have similar inhibitory properties though the compounds uniformly demonstrated greater potency against the 1b strains as compared to the 1a strains.

Specificity Assays

The specificity assays were performed to demonstrate the *in vitro* selectivity of the compounds of the present invention in inhibiting HCV NS3/4A protease complex as compared to other serine or cysteine proteases.

The specificities of compounds of the present invention were determined against a variety of serine proteases: human neutrophil elastase (HNE), porcine pancreatic elastase (PPE) and human pancreatic chymotrypsin and one cysteine protease: human liver cathepsin B. In all cases a 96-well plate format protocol using colorimetric p-nitroaniline (pNA) substrate specific for each enzyme was used as described previously (PCT Patent Application No. WO 00/09543) with some modifications to the serine protease assays. All enzymes were purchased from Sigma while the substrates were from Bachem.

Each assay included a 2 h enzyme-inhibitor pre-incubation at room temperature followed by addition of substrate and hydrolysis to ~30% conversion as measured on a Spectramax Pro microplate reader. Compound concentrations varied from 100 to 0.4 μ M depending on their potency.

The final conditions for each assay were as follows:

50 mM Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) pH 8, 0.5 M Sodium Sulfate (Na₂SO₄), 50 mM NaCl, 0.1 mM EDTA, 3% DMSO, 0.01% Tween-20 with:

133 μ M succ-AAA-pNA and 20 nM HNE or 8 nM PPE; 100 μ M succ-AAPF-pNA and 250 pM Chymotrypsin.

100 mM NaHPO₄ (Sodium Hydrogen Phosphate) pH 6, 0.1 mM EDTA, 3%
5 DMSO, 1 mM TCEP (Tris(2-carboxyethyl)phosphine hydrochloride), 0.01% Tween-20, 30 μ M Z-FR-pNA and 5 nM Cathepsin B (enzyme stock activated in buffer containing 20 mM TCEP before use).

The percentage of inhibition was calculated using the formula:

10

$$[1 - ((UV_{inh} - UV_{blank}) / (UV_{ctl} - UV_{blank}))] \times 100$$

A non-linear curve fit was applied to the inhibition-concentration data, and the 50% effective concentration (IC₅₀) was calculated by the use of Excel XI-fit
15 software.

HCV Replicon Cell-based Assay

An HCV replicon whole cell system was established as described by Lohmann V, Korner F, Koch J, Herian U, Theilmann L, Bartenschlager R., Science
20 285(5424):110-3 (1999). This system enabled us to evaluate the effects of our HCV Protease compounds on HCV RNA replication. Briefly, using the HCV strain 1b sequence described in the Lohmann paper (Assession number:AJ238799), an HCV cDNA was generated encoding the 5' internal ribosome entry site (IRES), the neomycin resistance gene, the EMCV (encephalomyocarditis viurs)-IRES and the
25 HCV nonstructural proteins, NS3-NS5B, and 3' non-translated region (NTR). In vitro transcripts of the cDNA were transfected into the human hepatoma cell line, Huh7. Selection for cells constitutively expressing the HCV replicon was achieved in the presence of the selectable marker, neomycin (G418). Resulting cell lines were characterized for positive and negative strand RNA production and protein
30 production over time.

Huh7 cells, constitutively expressing the HCV replicon, were grown in Dulbecco's Modified Eagle Media (DMEM) containing 10% Fetal calf serum (FCS)

and 1 mg/ml G418 (Gibco-BRL). Cells were seeded the night before (1.5×10^4 cells/well) in 96-well tissue-culture sterile plates. Compound and no compound controls were prepared in DMEM containing 4% FCS, 1:100 Penicillin / Streptomycin, 1:100 L-glutamine and 5% DMSO in the dilution plate (0.5% DMSO final concentration in the assay). Compound / DMSO mixes were added to the cells and incubated for 4 days at 37°C. After 4 days, plates were rinsed thoroughly with Phosphate-Buffered Saline (PBS) (3 times 150 µl). The cells were lysed with 25 µl of a lysis assay reagent containing the FRET peptide (RET S1, as described for the in vitro enzyme assay). The lysis assay reagent was made from 5X cell Luciferase cell culture lysis reagent (Promega #E153A) diluted to 1X with distilled water, NaCl added to 150 mM final, the FRET peptide diluted to 10 µM final from a 2 mM stock in 100% DMSO. The plate was then placed into the Cytofluor 4000 instrument which had been set to 340 nm excitation / 490 nm emission, automatic mode for 21 cycles and the plate read in a kinetic mode. EC₅₀ determinations were carried out as described for the IC₅₀ determinations.

Two different secondary assays were used to confirm EC₅₀ determinations from the replicon FRET assay. These included a quantitative RNA assay and a transient luciferase cell reporter assay. For the quantitative RNA assay, compound / no compound controls were incubated with the cells as described for the replicon FRET assay. After 4 days, cells were lysed using the Rneasy kit (Qiagen). Purified total RNA was normalized using RiboGreen (Jones LJ, Yue ST, Cheung CY, Singer VL, Anal. Chem., 265(2):368-74 (1998)) and relative quantitation of HCV RNA expression assessed using the Taqman procedure (Kolykhalov AA, Mihalik K, Feinstone SM, Rice CM, Journal of Virology 74, 2046-2051 (2000)) and the Platinum Quantitative RT-PCR Thermoscript One-Step kit (Invitrogen cat # 11731-015). Briefly, RNA made to a volume of 5 µl (≤ 1 ng) was added to a 20 µl Ready-Mix containing the following: 1.25X Thermoscript reaction mix (containing Magnesium Sulfate and 2-deoxynucleoside 5'-triphosphates (dNTPs)), 3 mM dNTPs, 200 nM forward primer (sequence: 5'-gggagagccatagtggctctgc-3'), 600 nM reverse primer (5'-cccaaattctccaggcattga-3'), 100 nM probe (5'-6-FAM-cggaattgccaggacgaccgg-BHQ-1-3')(FAM: Fluorescein-aminohexyl amidite; BHQ:

Black Hole Quencher), 1 μ M Rox reference dye (Invitrogen cat # 12223-012) and Thermoscript Plus Platinum Taq polymerase mixture. All primers were designed with ABI Prism 7700 software and obtained from Biosearch Technologies, Novato, CA. Samples containing known concentrations of HCV RNA transcript were run as standards. Using the following cycling protocol (50°C, 30 min; 95°C, 5 min; 40 cycles of 95°C, 15 seconds (s), 60°C, 1 min), HCV RNA expression was quantitated as described in the Perkin Elmer manual using the ABI Prism 7700 Sequence Detector.

The luciferase reporter assay was also used to confirm compound potency in the replicon. Utilization of a replicon luciferase reporter assay was first described by Krieger et al (Krieger N, Lohmann V, and Bartenschlager R, J. Virol. 75(10):4614-4624 (2001)). The replicon construct described for our FRET assay was modified by replacing the resistance gene neomycin with the Blasticidin-resistance gene fused to the N-terminus of the humanized form of Renilla luciferase (restriction sites AscI / PmeI used for the subcloning). The adaptive mutation at position 1179 (serine to isoleucine) was also introduced (Blight KJ, Kolykhalov, AA, Rice, CM, Science 290(5498):1972-1974). The luciferase reporter assay was set up by seeding huh7 cells the night before at a density of 2×10^6 cells per T75 flask. Cells were washed the next day with 7.5 ml Opti-MEM. Following the Invitrogen protocol, 40 μ l DMRIE-C was vortexed with 5 ml Opti-MEM before adding 5 μ g HCV reporter replicon RNA. The mix was added to the washed huh7 cells and left for 4 h at 37°C. In the mean time, serial compound dilutions and no compound controls were prepared in DMEM containing 10% FCS and 5% DMSO in the dilution plate (0.5% DMSO final concentration in the assay). Compound / DMSO mixes were added to each well of a 24-well plate. After 4 h, the transfection mix was aspirated, and cells washed with 5 ml of Opti-MEM before trypsinization. Trypsinized cells were resuspended in 10% DMEM and seeded at 2×10^4 cells/well in the 24-well plates containing compound or no compound controls. Plates were incubated for 4 days. After 4 days, media was removed and cells washed with PBS. 100 μ l 1x Renilla Luciferase Lysis Buffer (Promega) was immediately added to each well and the plates either frozen at -80°C for later analysis, or assayed after 15 min of lysis. Lysate

(40 μ l) from each well was transferred to a 96-well black plate (clear bottom) followed by 200 μ l 1x Renilla Luciferase assay substrate. Plates were read immediately on a Packard TopCount NXT using a luminescence program.

- 5 The percentage inhibition was calculated using the formula below:

$$\% \text{ control} = \frac{\text{average luciferase signal in experimental wells (+ compound)}}{\text{average luciferase signal in DMSO control wells (- compound)}}$$

- 10 The values were graphed and analyzed using XLFit to obtain the EC₅₀ value.

Biological Examples

- Representative compounds of the invention were assessed in the HCV replicon cell assay described above and/or in several of the outlined specificity assays described above. For example, Compound 9 was found to have an IC₅₀ of 128 nM against the BMS strain of the NS3/4A protease complex in the enzyme assay described above. Similar potency values were obtained with the published H77 (IC₅₀ of 62 nM) and J4L6S (IC₅₀ of 41 nM) strains. The EC₅₀ value in the replicon assay was 403 nM. In the specificity assays, Compound 9 was found to have the following activity: HNE = 46 μ M; PPE > 200 μ M; Chymotrypsin > 200 μ M; Cathepsin B > 50 μ M (assay interference at higher concentrations). These results indicate this family of compounds are highly specific for the NS3 protease and have utility for inhibiting HCV replicon replication.

25

- The compounds inhibited below were tested for biological activity according to the HCV replicon cell assay described above and were found to have activities set forth in Table 2 below. The activity ranges were classified into the following groups: for IC₅₀, and EC₅₀, A (least active) >1.5 μ M; B 0.15–1.5 μ M; C (most active) < 0.15 μ M. Preferably, the IC₅₀ values are from about 0.001-1 μ M, and most preferably less than 0.1 μ M. Preferably, the EC₅₀ values are from about 0.001 to 25
- 30

μM , more preferably from about 0.001-1 μM and most preferably less than about 0.1 μM .

| Cmpd | Activity Range IC50 | Activity Range EC50 |
|------|------------------------|------------------------|
| 1 | C | C |
| 2 | C | B |
| 3 | C | C |
| 4 | C | B |
| 5 | C | C |
| 6 | C | B |
| 7 | C | B |
| 8 | C | B |
| 9 | C | B |
| 10 | C | B |
| 11 | B | A |
| 12 | B | |
| 13 | B | A |
| 14 | C | C |
| 15 | C | C |
| 16 | C | C |
| 17 | C | B |
| 18 | C | C |
| 19 | C | C |
| 20 | C | C |
| 21 | B | B |
| 22 | B | |
| 23 | C | C |

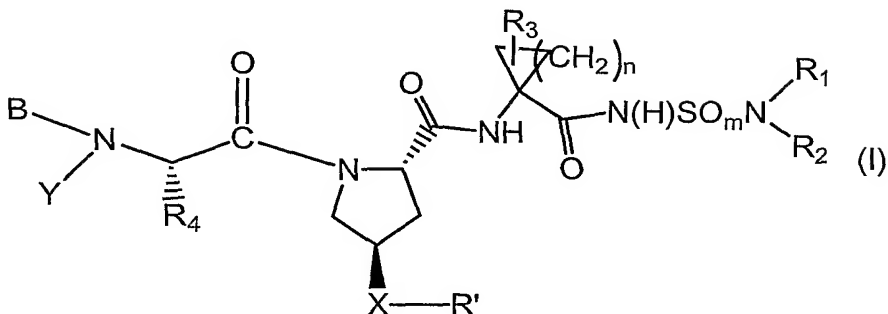
Although the invention has been described with respect to specific aspects, those skilled in the art will recognize that other aspects are intended to be included

within the scope of the claims which follow. Further, the disclosures of documents referenced herein, e.g., U.S. Patent No. 6,323,180 and U.S. Patent Application Publication No. 20020111313, are incorporated herein, in their entirety, by reference.

CLAIMS

1. A compound having the formula

5



wherein:

(a) R₁ and R₂ are each independently C₁₋₈ alkyl, C₃₋₇ cycloalkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, C₉₋₁₄ cycloalkylaryl, C₇₋₁₄ alkoxyaryl, C₉₋₁₄ cycloalkoxyaryl, 5-7 membered heteroaryl or C₇₋₁₄ alkylheteroaryl; or R₁ and R₂, together with the nitrogen atom to which they are attached, join to form a 4-8 membered monocyclic heterocycle;

(b) m is 1 or 2;

(c) n is 1 or 2;

(d) R₃ is H; or C₁₋₆ alkyl, C₂₋₆ alkenyl or C₃₋₇ cycloalkyl, each optionally substituted with halogen;

(e) R₄ is C₁₋₈ alkyl optionally substituted with halo, cyano, amino, C₁₋₆ dialkylamino, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, C₁₋₆ alkoxy, carboxy, hydroxy, aryloxy, C₇₋₁₄ alkylaryloxy, C₂₋₆ alkylester or C₈₋₁₅ alkylarylester; C₃₋₁₂ alkenyl; C₃₋₇ cycloalkyl or C₄₋₁₀ alkylcycloalkyl, wherein the cycloalkyl or alkylcycloalkyl are optionally substituted with hydroxy, C₁₋₆ alkyl, C₂₋₆ alkenyl or C₁₋₆ alkoxy; or R₃ together with the carbon atom to which it is attached forms a C₃₋₇ cycloalkyl group optionally substituted with C₂₋₆ alkenyl;

- (f) Y is H, phenyl substituted with nitro, pyridyl substituted with nitro, or C₁₋₆ alkyl optionally substituted with cyano, hydroxyl or C₃₋₇ cycloalkyl; provided that if R₅ or R₆ is H then Y is H;
- (g) B is H, C₁₋₆ alkyl, R₅-(C=O)-, R₅O(C=O)-, R₅-N(R₆)-C(=O)-, R₅-N(R₆)-C(=S)-, R₅SO₂-, or R₅-N(R₆)-SO₂-;
- (h) R₅ is (i) C₁₋₁₀ alkyl optionally substituted with phenyl, carboxyl, C₁₋₆ alkanoyl, 1-3 halogen, hydroxy, -OC(O)C₁₋₆ alkyl, C₁₋₆ alkoxy, amino optionally substituted with C₁₋₆ alkyl, amido, or (lower alkyl) amido; (ii) C₃₋₇ cycloalkyl, C₃₋₇ cycloalkoxy, or C₄₋₁₀ alkylcycloalkyl, each optionally substituted with hydroxy, carboxyl, (C₁₋₆ alkoxy)carbonyl, amino optionally substituted with C₁₋₆ alkyl, amido, or (lower alkyl) amido; (iii) C₆₋₁₀ aryl or C₇₋₁₆ arylalkyl, each optionally substituted with C₁₋₆ alkyl, halogen, nitro, hydroxy, amido, (lower alkyl) amido, or amino optionally substituted with C₁₋₆ alkyl; (iv) Het; (v) bicyclo(1.1.1)pentane; or (vi) -C(O)OC₁₋₆ alkyl, C₂₋₆alkenyl or C₂₋₆alkynyl;
- (i) R₆ is H; C₁₋₆ alkyl optionally substituted with 1-3 halogens; or C₁₋₆ alkoxy provided R₅ is C₁₋₁₀ alkyl;
- (j) X is O, S, SO, SO₂, OCH₂, CH₂O or NH;
- (k) R' is Het, C₆₋₁₀ aryl or C₇₋₁₄ alkylaryl, each optionally substituted with R^a; and
- (l) R^a is C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₁₋₆ alkoxy, C₃₋₇ cycloalkoxy, halo-C₁₋₆ alkyl, CF₃, mono-or di- halo-C₁₋₆ alkoxy, cyano, halo, thioalkyl, hydroxy, alkanoyl, NO₂, SH, , amino, C₁₋₆ alkylamino, di (C₁₋₆) alkylamino, di (C₁₋₆) alkylamide, carboxyl, (C₁₋₆) carboxyester, C₁₋₆ alkylsulfone, C₁₋₆ alkylsulfonamide, di (C₁₋₆) alkyl(alkoxy)amine, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, or a 5-7 membered monocyclic heterocycle;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

30

2. The compound of claim 1 wherein R₁ and R₂ are each independently C₁₋₈ alkyl, C₃₋₇ cycloalkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, 5-7 membered heteroaryl or C₇₋₁₄ alkylheteroaryl; or R₁ and R₂, together with the

nitrogen atom to which they are attached, join to form a 4-8 membered monocyclic heterocycle.

3. The compound of claim 2 wherein R_1 and R_2 are each independently C_{1-8} alkyl, C_{4-10} alkylcycloalkyl, C_{1-6} alkoxy, C_{6-10} aryl, C_{7-14} alkylaryl, 5-7 membered heteroaryl or C_{7-14} alkylheteroaryl, or R_1 and R_2 , together with the nitrogen atom to which they are attached, join to form a 5-6 membered monocyclic heterocycle.
4. The compound of Claim 1 wherein R_3 is C_{1-6} alkyl, C_{2-6} alkenyl or C_{3-7} cycloalkyl.
5. The compound of Claim 4 wherein R_3 is C_{2-6} alkenyl.
6. The compound of Claim 1 wherein R_4 is C_{1-8} alkyl optionally substituted with C_6 aryl, C_{1-6} alkoxy, carboxy, hydroxy, aryloxy, C_{7-14} alkylaryloxy, C_{2-6} alkylester or C_{8-15} alkylarylester; C_{3-12} alkenyl; C_{3-7} cycloalkyl; or C_{4-10} alkylcycloalkyl.
7. The compound of Claim 6 wherein R_4 is C_{1-8} alkyl optionally substituted with C_{1-6} alkoxy; or C_{3-7} cycloalkyl.
8. The compound of Claim 1 wherein Y is H.
9. The compound of Claim 1 wherein B is H, C_{1-6} alkyl, $R_5-(C=O)-$, $R_5O(C=O)-$, $R_5-N(R_6)-C(=O)-$, $R_5-N(R_6)-C(=S)-$, R_5SO_2- , or $R_5-N(R_6)-SO_2-$.
10. The compound of Claim 9 wherein B is $R_5-(C=O)-$, $R_5O(C=O)-$, or $R_5-N(R_6)-C(=O)-$.
11. The compound of Claim 10 wherein B is $R_5O(C=O)-$ and R_5 is C_{1-6} alkyl.
12. The compound of Claim 1 wherein R_5 is (i) C_{1-10} alkyl optionally substituted

with phenyl, carboxyl, C₁₋₆ alkanoyl, 1-3 halogen, hydroxy, C₁₋₆ alkoxy; (ii) C₃₋₇ cycloalkyl, C₃₋₇ cycloalkoxy, or C₄₋₁₀ alkylcycloalkyl; or (iii) C₆₋₁₀ aryl or C₇₋₁₆ arylalkyl, each optionally substituted with C₁₋₆ alkyl or halogen.

- 5 13. The compound of Claim 12 wherein R₅ is (i) C₁₋₁₀ alkyl optionally substituted with 1-3 halogen or C₁₋₆ alkoxy; or (ii) C₃₋₇ cycloalkyl or C₄₋₁₀ alkylcycloalkyl.

14. The compound of Claim 1 wherein R₆ is H or C₁₋₆ alkyl optionally substituted
10 with 1-3 halogens.

15. The compound of Claim 14 wherein R₆ is H.

16. The compound of Claim 1 wherein X is O or NH.
15

17. The compound of Claim 1 wherein R' is Het; or C₆₋₁₀ aryl optionally substituted with R^a.

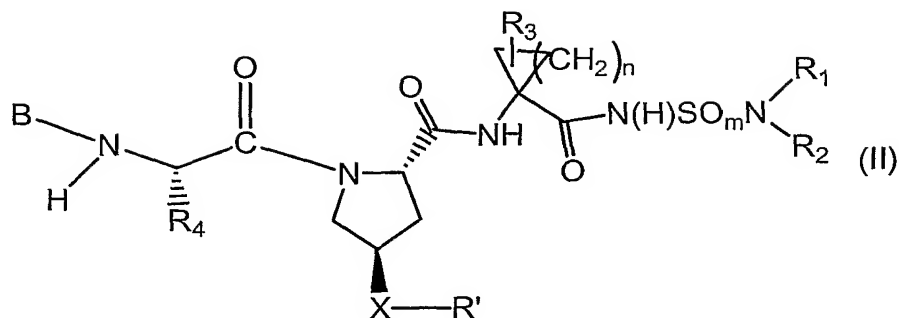
18. The compound of Claim 17 wherein R' is Het.
20

19. The compound of Claim 18 wherein the heterocycle contains 1 or 2 nitrogen atoms and optionally a sulfur atom or an oxygen atom in the ring.

20. The compound of Claim 19 wherein the heterocycle is substituted with at
25 least one of C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, or a 5-7 membered monocyclic heterocycle.

21. The compound of Claim 1 wherein R^a is C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₁₋₆ alkoxy, halo-C₁₋₆ alkyl, halo, amino, C₆ aryl, or a 5-7 membered monocyclic
30 heterocycle.

22. A compound having the formula



wherein:

- (a) R₁ and R₂ are each independently C₁₋₈ alkyl, C₃₋₇ cycloalkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, 5-7 membered heteroaryl or C₇₋₁₄ alkylheteroaryl; or R₁ and R₂, together with the nitrogen atom to which they are attached, join to form a 4-8 membered monocyclic heterocycle;
- (b) m is 1 or 2;
- (c) n is 1 or 2;
- (d) R₃ is C₁₋₆ alkyl, C₂₋₆ alkenyl or C₃₋₇ cycloalkyl;
- (e) R₄ is C₁₋₈ alkyl optionally substituted with C₆ aryl, C₁₋₆ alkoxy, carboxy, hydroxy, aryloxy, C₇₋₁₄ alkylaryloxy, C₂₋₆ alkylester, C₈₋₁₅ alkylarylester; C₃₋₁₂ alkenyl; C₃₋₇ cycloalkyl; or C₄₋₁₀ alkylcycloalkyl;
- (f) B is H, C₁₋₆ alkyl, R₅-(C=O)-, R₅O(C=O)-, R₅-N(R₆)-C(=O)-, R₅-N(R₆)-C(=S)-, R₅SO₂-, or R₅-N(R₆)-SO₂-;
- (g) R₅ is (i) C₁₋₁₀ alkyl optionally substituted with phenyl, carboxyl, C₁₋₆ alkanoyl, 1-3 halogen, hydroxy, C₁₋₆ alkoxy; (ii) C₃₋₇ cycloalkyl, C₃₋₇ cycloalkoxy, or C₄₋₁₀ alkylcycloalkyl; or (iii) C₆₋₁₀ aryl or C₇₋₁₆ arylalkyl, each optionally substituted with C₁₋₆ alkyl or halogen;
- (h) R₆ is H or C₁₋₆ alkyl optionally substituted with 1-3 halogens;
- (i) X is O or NH;
- (j) R' is Het; or C₆₋₁₀ aryl optionally substituted with R^a; and
- (k) R^a is C₁₋₆ alkyl, C₃₋₇ cycloalkyl, C₁₋₆ alkoxy, halo-C₁₋₆ alkyl, halo, amino, C₆ aryl, or a 5-7 membered monocyclic heterocycle;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

23. The compound of claim 22 wherein R₁ and R₂ are each independently C₁₋₈
5 alkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, 5-7 membered
heteroaryl, C₇₋₁₄ alkylheteroaryl; or R₁ and R₂, together with the nitrogen atom to
which they are attached, join to form a 5-6 membered monocyclic heterocycle

24. The compound of claim 23 wherein R₁ and R₂ are each independently C₁₋₃
10 alkyl or C₁₋₃ alkoxy.

25. The compound of Claim 22 wherein R' is a bicyclic heterocycle.

26. The compound of Claim 25 wherein the heterocycle contains 1 or 2 nitrogen
15 atoms and optionally a sulfur atom or an oxygen atom in the ring.

27. The compound of Claim 25 wherein the heterocycle is substituted with at
least one of C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, C₆ aryl and a 5-7 membered monocyclic
heterocycle.

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28. The compound of Claim 22 wherein R' is a bicyclic heterocycle containing 1
nitrogen atom and substituted with methoxy and at least one of a C₆ aryl and a 5-7
membered monocyclic heterocycle.

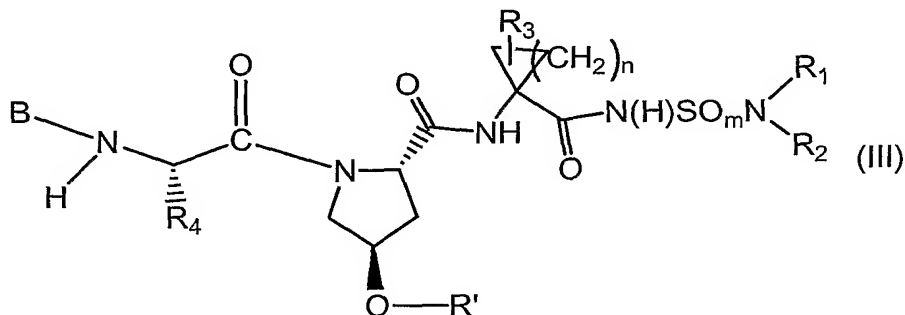
25 29. The compound of Claim 22 wherein R' is a monocyclic heterocycle.

30. The compound of Claim 29 wherein the heterocycle contains 1 or 2 nitrogen
atoms and optionally a sulfur atom or an oxygen atom in the ring.

30 31. The compound of Claim 29 wherein the heterocycle is substituted with at
least one of C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, or a 5-7 membered
monocyclic heterocycle.

32. The compound of Claim 22 wherein R' is a monocyclic heterocycle containing 1 or 2 nitrogen atoms and substituted with methoxy and at least one of a C₆ aryl and a 5-7 membered monocyclic heterocycle.

5 33. A compound having the formula



wherein:

- 10 (a) R₁ and R₂ are each independently C₁₋₈ alkyl, C₄₋₁₀ alkylcycloalkyl, C₁₋₆ alkoxy, C₆₋₁₀ aryl, C₇₋₁₄ alkylaryl, 5-7 membered heteroaryl or C₇₋₁₄ alkylheteroaryl; or R₁ and R₂, together with the nitrogen atom to which they are attached, join to form a 5-6 membered monocyclic heterocycle;
- 15 (b) m is 1 or 2;
- (c) n is 1 or 2;
- (d) R₃ is C₂₋₆ alkenyl;
- (e) R₄ is C₁₋₈ alkyl;
- (f) B is R₅O(C=O)-, or R₅-NH-C(=O)-;
- (g) R₅ is C₁₋₁₀ alkyl;
- 20 (h) R' is a bicyclic heterocycle optionally substituted with R^a; and
- (i) R^a is C₁₋₆ alkyl, C₁₋₆ alkoxy, halo, C₆ aryl, or a 5-7 membered monocyclic heterocycle;

or a pharmaceutically acceptable salt, solvate or prodrug thereof.

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34. The compound of claim 33 wherein R₁ and R₂ are each independently C₁₋₃ alkyl or C₁₋₃ alkoxy.

35. The compound of claim 34 wherein R₁ is methyl.
36. The compound of claim 34 wherein R₂ is methyl or methoxy.
- 5 37. The compound of Claim 33 wherein R₃ is vinyl.
38. The compound of Claim 33 wherein R₄ is t-butyl.
39. The compound of Claim 33 wherein R₅ is t-butyl.
- 10 40. The compound of Claim 33 wherein R' is quinoline or isoquinoline optionally substituted with R^a.
41. The compound of Claim 31 wherein R₁ is methyl, R₂ is methoxy, R₃ is vinyl,
15 R₄ is t-butyl, R₅ is t-butyl, and R' is isoquinoline substituted with at least one R^a.
42. The compound of Claim 41 wherein R^a is C₁₋₆ alkoxy.
43. The compound of Claim 42 wherein R^a further includes at least one of C₆ aryl
20 or a 5-7 membered monocyclic heterocycle.
44. A composition comprising the compound of Claim 1 and a pharmaceutically acceptable carrier.
- 25 45. The composition according to Claim 44 further comprising another compound having anti-HCV activity.
46. The composition according to Claim 45 wherein the other compound having anti-HCV activity is an interferon.
- 30 47. The composition according to Claim 46 wherein the interferon is selected from the group consisting of interferon alpha 2B, pegylated interferon alpha, consensus interferon, interferon alpha 2A and lymphoblastiod interferon tau.

48. The composition according to Claim 45 wherein the other compound having anti-HCV activity is selected from the group consisting of interleukin 2, interleukin 6, interleukin 12, a compound that enhances the development of a type 1 helper T cell response, interfering RNA, anti-sense RNA, Imiquimod, ribavirin, an inosine 5'-monophosphate dehydrogenase inhibitor, amantadine, and rimantadine.
49. The composition according to the Claim 44 further comprising an interferon and ribavirin.
50. The composition according to Claim 45 wherein the compound having anti-HCV activity is a small molecule compound.
51. The composition according to Claim 50 wherein the compound having anti-HCV activity is effective to inhibit the functioning of a target selected from the group consisting of HCV metalloprotease, HCV serine protease, HCV polymerase, HCV helicase, HCV NS4B protein, HCV entry, HCV assembly, HCV egress, HCV NS5A protein, IMPDH and a nucleoside of HCV.
52. A method of inhibiting the functioning of the HCV serine protease comprising contacting the protease with the compound of Claim 1.
53. A method of treating an HCV infection in a patient, comprising administering to the patient a therapeutically effective amount of the compound of Claim 1, or a pharmaceutically acceptable solvate, prodrug or salt thereof.
54. The method according to Claim 53 wherein the compound is effective to inhibit the functioning of the HCV serine protease.
55. The method according to Claim 53 further comprising administering another compound having anti-HCV activity prior to, after or simultaneously with the compound of Claim 1.

56. The method according to Claim 55 wherein the other compound having anti-HCV activity is an interferon.
57. The method according to Claim 56 wherein the interferon is selected from
5 the group consisting of interferon alpha 2B, pegylated interferon alpha, consensus interferon, interferon alpha 2A, lymphoblastoid interferon tau.
58. The method according to Claim 55 wherein the other compound having anti-HCV activity is selected from the group consisting of interleukin 2, interleukin 6,
10 interleukin 12, a compound that enhances the development of a type 1 helper T cell response, interfering RNA, anti-sense RNA, Imiquimod, ribavirin, an inosine 5'-monophosphate dehydrogenase inhibitor, amantadine, and rimantadine.
59. The method according to Claim 55 wherein the other compound having anti-
15 HCV activity is a small molecule.
60. The method according to Claim 59 wherein the compound having anti-HCV activity is effective to inhibit the functioning of a target selected from the group consisting of HCV metalloprotease, HCV serine protease, HCV polymerase, HCV
20 helicase, HCV NS4B protein, HCV entry, HCV assembly, HCV egress, HCV NS5A protein, IMPDH and a nucleoside of HCV.
61. The method according to Claim 59 wherein the other compound having anti-HCV activity is effective to inhibit the functioning of target in the HCV life cycle
25 other than HCV serine protease.
62. Use of the compound of Claim 1 for the manufacture of a medicament for treating HCV infection in a patient.
- 30 63. Use of the composition of Claim 44 for the manufacture of a medicament for treating HCV infection in a patient.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US04/38165

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : A61K 38/06; C07K 5/08

US CL : 514/2; 530/300

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/2,18; 530/300,331

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
STN for compound, EAST, PubMed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|--|-----------------------|
| X | US 6,642,204 B2 (Lilias-Bruner et al) 4 November 2003 (04.11.2003), See entire document especially abstract and columns 3 and 4. | 1-63 |
| X,P | US 2004/0077551 A1 (Campbell et al) 22 April 2004 (22.04.2004), see entire document, especially column 4. | 1-63 |

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

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- "O" document referring to an oral disclosure, use, exhibition or other means
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- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
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Date of the actual completion of the international search

03 March 2005 (03.03.2005)

Date of mailing of the international search report

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